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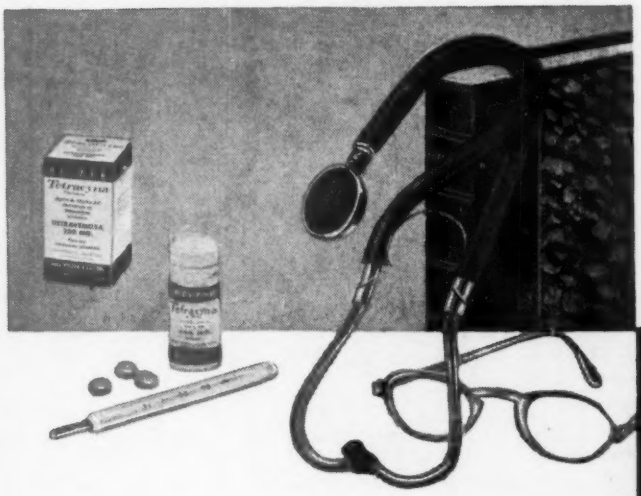

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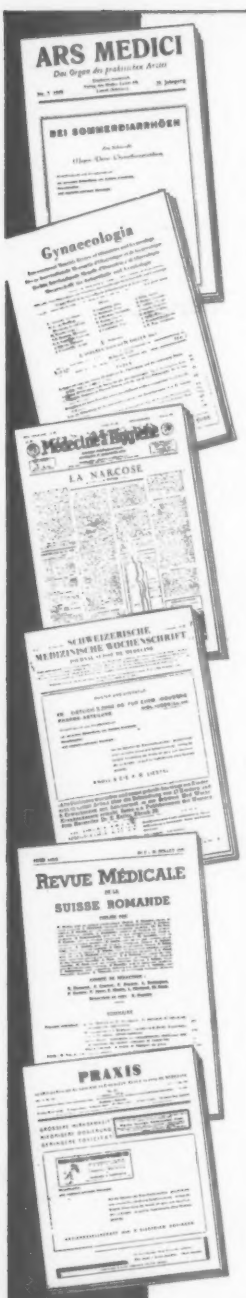

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Buenos Aires - Argentina

EFFECTS OF DECAMETHONIUM (C-10) ON THE NEURO-MUSCULAR JUNCTION OF SOLEUS IN THE CAT *

F. HUIDOBRO

(Department of Pharmacology, Catholic University, Santiago de Chile)

ACCORDING TO present knowledge, there are three types of subtances which cause neuro-muscular blocking: a) those which prevent the depolarizing action of the chemical transmitter; b) those which depolarize the motor end-plate, and c) those which may polarize the synaptic area. Groups *a* and *b* were directly demonstrated by electrical measurements (1). Group *c* was indirectly induced through pharmacological tests (6). In addition to these three groups, an intermediary between *a* and *b* has been suggested (19). Compounds belonging to this fourth group have a double action: first they depolarize the motor end-plate and at a certain moment of the paralysis, they prevent depolarization of the transmitter. Decamethonium (C-10) is the characteristic substance of this sub-group (19) and that is its mechanism of action on the cat soleus, meanwhile in other muscles of the same animal it has only a pure depolarizing action (8, 13, 20, 21).

The drug may be joined to the receptor through intermolecular forces (3). It is probable that the bis-ammonium quaternary bases, both natural and synthetic, having the property of causing neuro-muscular paralysis, are joined to the receptor through these forces, because the active parts of the molecules are found separated by almost similar distances (2, 4, 9, 10, 16).

Moreover, other experimental data suggest that the electric charge of quaternary ammonia is responsible for the type of block produced by these compounds (14, 15, 16, 17, 18). According to these considerations, it is difficult to imagine how a molecule joined to the receptor zone of the cells may change the type of block; i. e., observing at the beginning depolarization of the motor end-plate and at a determined moment of the block, pre-

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vention of the depolarizing action of acetylcholine. This theoretical difficulty led us to investigate if C-10 may have the double action described above.

METHODS

Non-selected adult cats were employed, under chloralose anesthesia (80 mg per Kg of body weight) injected into the femoral vein.

Experiments were performed mainly on the soleus muscle but also on the anterior tibialis. The tendons of the muscle being tested were attached to rubber bands; the bones were fixed at the same time. Muscular contractions were recorded on a kymograph. Electrical stimuli were applied through the nerve which was centraly crushed. A Grass stimulator (model S 4 A) was employed and stimuli were rectangular pulses of 0.1 ms duration and slightly supramaximal. The frequency of stimulation varied in accordance with the type of experiment. Silver electrodes insulated with rubber were employed. A cannula was inserted in the trachea to permit artificial respiration when necessary.

In some animals, an atrophied soleus was obtained by aseptic section of the tendon, 4 to 14 days before.

The drugs used were: decamethonium (C-10) (Burroughs Wellcome and Co) at doses between 1.5 and 100 μg; d-tubocurarine (d-TC) (Inst. Bioquímico Beta) at doses from 0.1 to 0.6 mg and neostigmine (Inst. Bioquímico Beta) at doses between 100 to 200 μg. These substances, in distilled water solution, were rapidly injected into the terminal portion of the abdominal aorta after ligation of the inferior mesenteric, medial sacral and contralateral iliac arteries.

RESULTS

I: *Effect of C-10 and dTC on tetanic stimulation of the muscle.* — Some of the experiments were performed using simultaneously the soleus (slow or red muscle) and the tibialis (rapid or white muscle) with the purpose of comparing the effect of drugs on them. In other cases, only the soleus was employed. Muscles were stimulated through the nerve at a frequency varying between 20 and 30 pulses per second, during 2 minutes alternating with 2 minutes of rest. After some stimulations muscular contractions become almost similar ([5]). Once this stable stage was reached, C-10 or dTC were administered. A comparison of the effects of drugs can be seen in fig. 1, which presents the result of an experiment in which the muscle was stimulated at a frequency of 30 pulses per second. The maximal tension and the height of the contraction were each considered as 100 %. The depression caused by the drug and the post-paralysis recovery was indicated in percent of the control period. Each point in the figure represents the maximal tension and also the maximal tension of the cessation of the stimulation. It may be observed that the responses of the soleus are not similar to those of the tibialis. When the tibialis was used, the post-paralysis recovery produced by C-10 and dTC was very rapid and the tension exceeded the level, especially the tension following cessation of the stimulus. The opposite occurred in the soleus. The post-paralysis recovery was slow, the tension never exceeded the control level. and sometimes a pheno-

menon similar to Wedensky's persisted for a long period, regardless of the drug employed (fig. 1).

When the soleus was stimulated with a lower frequency under which conditions the decrease in the tension is less, a clear difference in recovery after administration of C-10 and dCT can be observed. Fig. 2 illustrates a typical experiment which shows that tetanus is more poorly maintained when giving dTC than when giving C-10. On analyzing the figure, the response might be thought of as a summation of effects; but this does

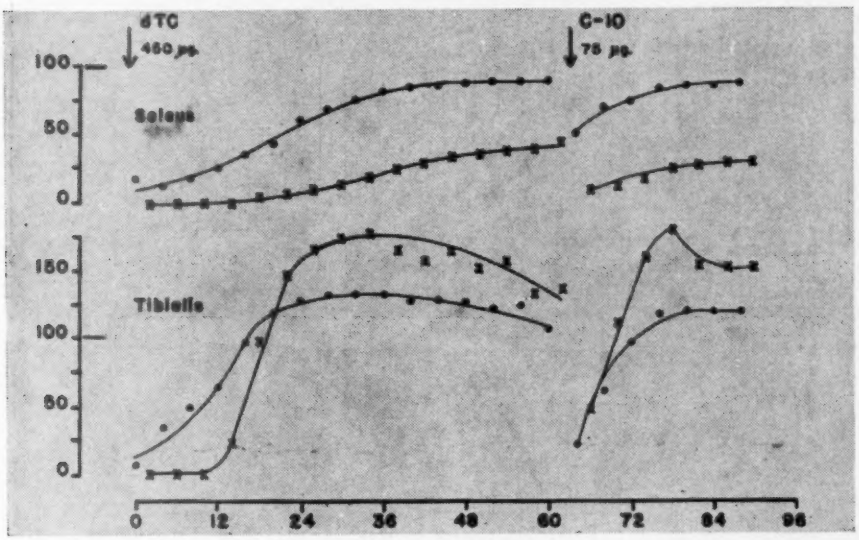

FIG. 1. — *Effect of dTC and C-10 on the tetanic contraction of the soleus and the tibialis.*
Cat weighing 2.900 g - Tetanic stimulation through the nerve, 30 pulses per second, for 2 minutes, alternating with 2 minutes of rest.
●— — — —● *maximal height of contraction.*
x— — — —x *height of contraction at the end of the stimulus.*
Abcissa: time in minutes: Ordinate: % of contraction during the control period. For details, see the text.

not seem to be the case, for on other occasions, dTC was injected before C-10, and the same above mentioned effects were observed. This phenomenon is not so evident in fig. 1, as it was under different experimental conditions.

The sensitivity of muscles to the action of the drugs is also different. Small doses of dTC depress first the tibialis. Larger doses produce paralysis of more or less the same intensity. Spontaneous recovery is more rapid in the tibialis. The same facts are observed when using C-10 (fig. 1).

II: *Effects of C-10 on muscular twitching, on the action of neostigmine and on the post- tetanus effect.* — The soleus muscle was used, stimulated through the nerve at 6 pulses per minute. In some cases, a

short tetanus (20 seconds) of 100 pulses per second was interposed. Small doses of C-10 were injected into the terminal portion of the abdominal aorta with the object of producing a slight depression of muscular twitching. If administration of C-10 was repeated several times, a more marked depression was produced in each occasion, as can be seen in fig. 3. Another

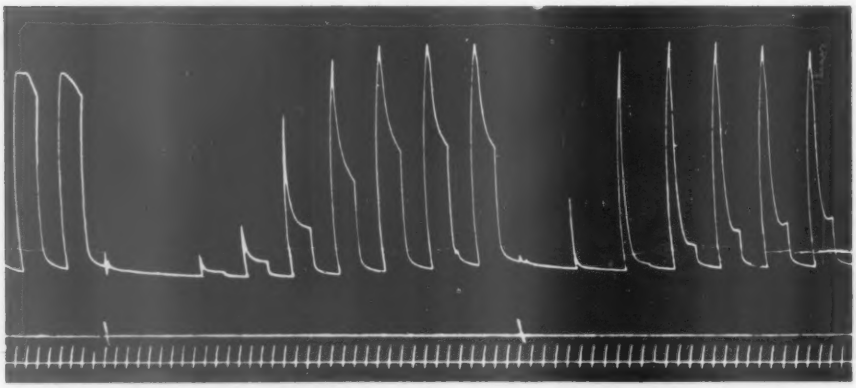

FIG. 2. — *Effects of C-10 and dTC on the tetanic stimulation of the soleus excited through the nerve (25 stimuli per second for 2 minutes).*
Cat weighing 2.850 g.
At first mark: C-10 (75 µg injected into the terminal portion of the abdominal aorta).
At second mark: aTC (450 µg injected into the terminal portion of the abdominal aorta).
Time in minutes.

important fact can be also observed in fig. 3: neostigmine does not modify the course of post-paralysis recovery; on the contrary, in many experiments, the block is augmented as may be seen in the figure.

A post-tetanic increase is not always present. Only in 5 out of 10 experiments has it been possible to find it. When it appears, it is of slight intensity and is only observed when the quantity of C-10 given is not able to depress 100 % the height of muscular twitching (fig. 3). In the same animal and under the same dose of C-10, a tetanus of the same frequency and duration may produce a slight increase of post-tetanic twitching or it may show no action. In this regard, there is a considerable difference of the post-tetanic effect when muscular twitching is depressed by dTC. Under these conditions, the post-tetanic effect is constant and important, and twitching may reappear although they are completely abolished by the action of the drug.

III: *Antagonism of C-10 against dTC and of dTC against C-10.* — Experiments were done stimulating the soleus through the nerve with 6 pulses per minute. A dose of C-10 was injected during stimulation in order to obtain control of paralysis. Immediately, dTC was given several times to know intensity and course of block. After obtaining the block and when recovery was almost complete, the same dose of C-10 was in-

jected again. As fig. 4 shows, the quantity of C-10 which produced depression of muscular twitching before the alkaloid, did not cause paralysis of muscular contractions after dTC and, furthermore, produced a small potentiation of the twitching. After this last administration of

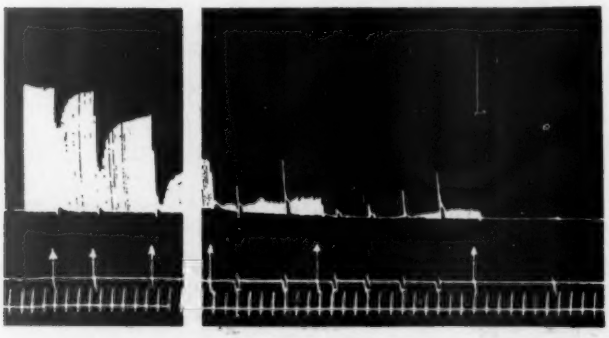

FIG. 3. — *Effect of successive doses of C-10, action of a tetanus and of neostigmine on the depression produced by C-10.*
Cat weighing 2.500 g - Muscle stimulated through the nerve at a frequency of 1 pulse every 10 seconds.
Arrows: C-10 (25 µg injected into the terminal portion of the abdominal aorta).
Marks: Tetanus of 20 seconds duration at a frequency of 100 per second.
Last mark: neostigmine (150 µg injected into the terminal portion of the abdominal aorta).
Between the first and second figure: 12 minutes and another injection of 25 µg of C-10.
Time in minutes.

C-10, the action of new doses of dTC caused disproportionally prolonged paralytic effects in relation with those produced by the drug given immediately after C-10 (Fig. 4). Antagonistic effects of C-10 against dTC may be better appreciated in fig. 5. Moreover, when twitching is depressed by dTC, paralysis is counteracted by C-10 in a definite form, as may be seen in fig. 4.

IV: *Effect of C-10 on the soleus atrophied by tenotomy.* — All the aforementioned effects produced by C-10 on the normal soleus may be produced also on the atrophied soleus but to a more intense degree.

DISCUSSION

Jewell and Zaimis (8) have reported that decamethonium has a double mechanism of action on the soleus muscle because the drug possesses the characteristics of depolarizing substances and also those of compounds preventing depolarization caused by the chemical transmitter. Thus, the block is preceded by potentiation of muscular twitching and counteracted by tetanus and by the action of neostigmine; tetanus is not well sustained

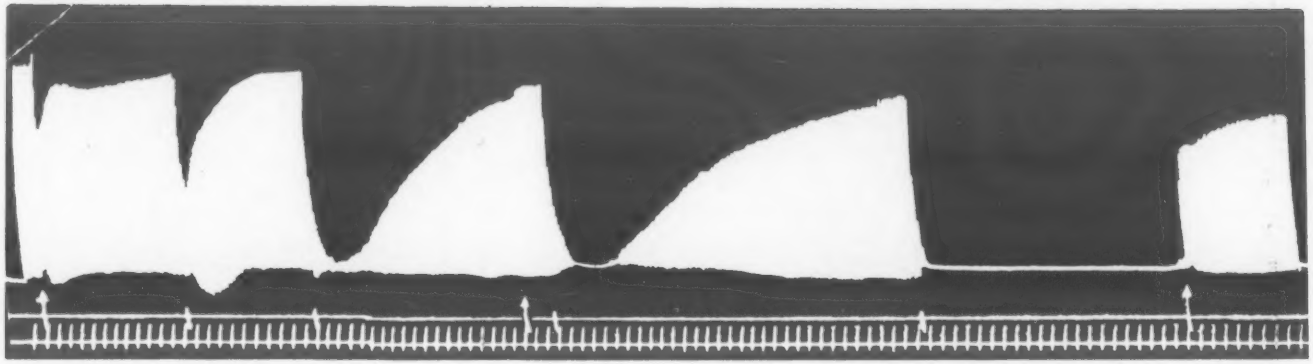

FIG. 4. — *Antagonism between dTC and C-10.*
Cat weighing 2.800 g - Soleus stimulated through the nerve at a frequency of 1 pulse every 10 seconds.
Arrows: C-10 (85 µg injected into the terminal portion of the abdominal aorta).
Marks: dTC (600 µg injected in the same way).
Time in minutes.

and paralysis increases by the action of dTC. Furthermore, successive doses of C-10 produce a phenomenon similar to tachyphylaxis.

Generally, our results are not in complete accordance with those obtained by Jewell and Zaimis (8). Only potentiation of muscular twitching by the action of small doses of C-10 has been confirmed. When comparing the action of a current producing tetanus and neostigmine on twitching of the soleus depressed by C-10 or dTC with the observations on the tibialis, a great difference can be appreciated. In the tibialis, post-tetanic recovery of muscular twitching depressed by dTC is evident and

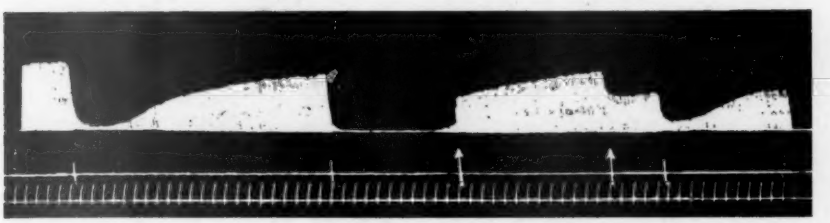

FIG. 5. — *Antagonism between C-10 and dTC.*
Cat weighing 3.760 g - Soleus stimulated through the nerve at a frequency of 1 pulse every 10 seconds.
Arrows: C-10 (100 µg injected into the abdominal aorta).
Marks: dTC (600 µg injected into the obdominal aorta).
Time in minutes.

important; after administration of neostigmine something similar occurs: this substance can counteract almost completely the depression produced by dTC. On the other hand, when there was 90 % block of muscular contractions, and only in these conditions (for if blocking is more intense, the phenomenon is not observed) or when paralysis begins to appear again, a tetanus can produce in some of the cases, a slight increase of twitching. If neostigmine is used when the animals have been given only C-10 and when its contractions are depressed 90 % or appear again after a more intense block, neostigmine cannot counteract the paralysis of the soleus; conversely, there are some cases in which depression appears more intense (only in one out of seven experiments, neostigmine slightly increased twitching).

In general, the soleus is a muscle incapable of sustaining tetanus when its contractions are depressed by the action of a blocking drug either C-10 or dTC (fig. 1). The tibialis is more able to sustain tetanus (fig. 1). Notwithstanding, when the frequency of the tetanic current is not so high, some differences can be observed. Fig. 2 shows how soleus sustains tetanus after administration of C-10 better than after administration of dTC.

The results herein discussed made us suspect that C-10 may have depolarizing properties. This possibility is reinforced by the following observations: a) dTC counteracts paralysis produced by C-10 (Fig. 4); b) C-10 decreases the effects caused by dTC and counteracts the paraly-

sis produced by the drug (fig. 4 and 5). This double antagonism may be only understood if one accepts that dTC may act by preventing the depolarizing action of acetylcholine and that C-10 only causes depolarization of the end-plate during all the time that the action persists. If this assumption were correct, it would agree with what we reported in the introduction of this paper, i. e., as neuromuscular block is under certain physical laws, it is not probable that the type of paralysis may change during its course. Then, it is more reasonable to accept that the peculiarity of each structure is what varies in front of the action of a pharmacological agent and not the properties of the drug itself.

When comparing the sensitivity of C-10 or dTC on the tibialis and soleus, both muscles being stimulated through the nerve by tetanic currents, it is evident that tibialis is far more deeply depressed than soleus by small doses of drugs. Recovery is much more rapid in the tibialis and may exceed the control value (fig. 1). Orrego and Luco ([11]) found higher sensitivity in the tibialis when employing frequencies of 0.5 pulses per second. We reached the same conclusion in our laboratory by indirect stimulation of the muscles of 1 pulse every 10 seconds, although larger doses of drugs produced a more prolonged effect on the soleus. All these facts are not in accordance with the results of Paton and Zaimis ([12]) as they found soleus much more senstive to the effect of dTC than the tibialis, but they agree that the tibialis has a more intense reaction to the effect of C-10 than the soleus. In addition, it was confirmed that post-paralysis recovery may exceed the control level when the tibialis is blocked with C-10; the same behaviour is observed when the quadriceps is depressed by C-10 or dTC ([5]).

The arguments suggesting that C-10 may have effects not only located on the motor endplate but also on the muscular fiber itself, have been summarized elsewhere ([5]). The experiments herewith reported suggest new places for the action of the drug. Hutter ([7]) reports that post-tetanic decurarization is due to a pre-synaptic phenomenon. The fact that post-tetanic recovery is not clearly observed when C-10 is being administered would indicate that the drug would act on the nervous ends preventing the increase of acetylcholine by post-tetanus nervous impulse.

SUMMARY

The effect of C-10 on the neuromuscular preparation of soleus and tibialis of cats under chloralose anesthesia was investigated. Its effects were compared with those produced by dTC. The findings are:

1) The tibialis sustained tetanus better than soleus regardless of the paralyzing agent employed: C-10 or dTC. When comparing the capacity of soleus to sustain tetanus after dTC or C-10, it was possible to observe that tetanus was not well sustained in the cases of dTC and was much better sustained when using C-10.

2) The same doses of C-10, repeated several times, produced every time more important depressions of the contractions of soleus; muscular twitching depressed by this drug was not counteracted by the effect of neostigmine. Post-tetanic recovery was scarce and was not observed in all the cases (fig. 3).

3) The soleus muscle showed a clear antagonism of C-10 against dTC and also of dTC against C-10.

4) The effects of C-10 were more intense in the soleus atrophied by tenotomy than in the normal muscle.

The results obtained are discussed and it is concluded that C-10 has only a depolarizing effect on the soleus of the cat.

Addendum: After this paper was prepared for publication, the more recent studies of Jewell and Zaimis appeared in the *J. Physiol.*, 1954, *124*, 417 and 429. They employed the same experimental methods and animals. Neostigmine was also given at the same doses although they made intravenous injections. Even though the experimental procedures were nearly the same, their results do not agree with those reported here. At this time it is not possible to explain the differences in results.

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ON THE PROBLEM OF THE ABSORPTION OF PROTAMINE-ZINC-INSULIN IMPLANTED SUBCUTANEOUSLY. PRESENCE OF BLOOD VESSELS IN THE CAPSULE.

L. VARGAS AND L. SILVA

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ACCORDING TO Gilliland and Martin (1951), there was no absorption of the protamine-zinc-insulin (P. Z. I.) implanted subcutaneously in the rabbit, and the pellets of P. Z. I. - cholesterol retained their initial weight after 3 months of implantation. On the contrary, in our previous experiments a complete absorption of the pellets was attained after 120 days. Moreover, Gilliland and Martin (1951) reported the absence of blood vessels in the subcutaneous capsule of Neutral P. Z. I. or P. Z. I. 50 % cholesterol, whereas Lewin (1948) and Silva (1951), described blood vessels around and inside the capsule of both substances. Biskind et al. (1941), Forber (1941) and Greenblatt (1943), have mentioned the presence of blood vessels in the surface of the capsule of steroid hormones implantation. However, Geist, Walter and Salmon (1940) observed only lymphatic vessels, and Vest and Howard (1939) and Hartman (1940) described the capsule without mentioning the existence of blood vessels.

The problem of the vascularity of the capsule and the penetration of the blood vessels inside of it seems of importance in the understanding of the absorption mechanism of the implanted material.

For these reasons and because of the contradictory results mentioned above, it was considered worthwhile to study again the absorption of P. Z. I. and the vascularity of the capsule. The present paper refers to a group of experiments devised with such a purpose, in animals as well as in human beings.

METHOD

We used sterile compounds of pure P. Z. I. plus cholesterol in a 1:1 proportion, with a variable weight ratio of 10 to 110 mg, implanted subcutaneously in the dorsal region of 148 rabbits and guinea pigs. The pellets

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were removed at different periods from 1 until 204 days of implantation. As a control, we studied 57 implantations of papaverine and 21 of cholesterol. The capsule originated around the pellets was studied microscopically

TABLE I

Frequency of fatal hypoglycemic coma after subcutaneous implantation of P. Z. I, during the first 48 hours

Animal	Body weight at implant minim/ max. g	Type of insulin °	Unit. per kg	Number of animals: implanted	with coma	% of coma	Minimal lethal dose	Maximal tolerated dose
Rabbit	1125-2250	HZI	184-935	10	3	20	315	935
	1300-1800	PZI-Bac	360-750	10	1			
	1500-3750	HZI-col	50-235	11	1	9	152	235
	1200-2850	NPH-col	170-650	20	12	60	170	600
	1600-2400	PZI-col	202-572	14	2	14	225	450
Guinea pig	300- 700	PZI-Ch	150-400	10	4	55	180	400
	290- 650	PZI-Sq	120-465	8	6		167	212
	200- 700	PZI-col	64-1200	38	17	45	160	940
	250- 480	PZI-col	148-298	20	3	15	186	298
	350- 725	HZI-col	83-228	7	0	—	—	228
		Control	mg/kg				*Days observation*	
Guinea pig	300- 700	Cholesterol	20- 200	21	0	0	5 to 130	
	325-1025	Papaverine	60-1720	57	0	0	1 to 120	

The first two series, from Lewin (1949) and the last one, partially from Melkonian (1950).

° HZI = histone-zinc-insulin Sanitas (Chile); HZI-col = idem with 50 % cholesterol; NPH-col = Neutral P. Z. I. with 50 % cholesterol (Canada); PZI-Bac = P. Z. I. Bacteriológico Chile; PZI-col = P. Z. I. prepared in our laboratory from crystalized insulin; PZI-Ch = P. Z. I. Choay (France); PZI-Sq = P. Z. I. Squibb (U.S.A.).

and the degree of development of the blood vessels was evaluated using crosses (Table II). In 10 guinea pigs, not included in this table, pellets of P. Z. I. plus 10 % of aluminum monoestearate were implanted. These

animals were injected, through the aorta, with Indian ink, from the 8th. day post-implantation until the 38th. Each histologic sample was stained with hematoxyline-eosin and Van Gieson. The blood sugar content was determined by the Shaffer-Hartmann's method, after a period of 12 hours fasting. Food employed was the standard diet used in the laboratory, with water *ad libitum*. In the diabetic patients the plan formerly indicated by Vargas (1949) was followed. In order to compare some of the results obtained with the subcutaneous implantations, the data on intravisceral implantations of Vargas and Maturana (1953) have been used in the present paper (fig. 3). These implantations were also done aseptically under ether anesthesia; the abdominal wall was opened and the pellet introducet into the liver or pancreas throughout a glass trocar.

RESULTS

a) *Vascularity of the capsule.*

We have carefully studied the vascularity of the capsule of 59 P. Z. I. and of 21 cholesterol implantations, removed at intervals from 1 to 204 days, taking into consideration the chronological reaction and the amount of blood vessels and their distribution (Table II). At first, when the capsule had not yet developed (1-5 days), no blood vessels of neoformation were found, but from the fifth day of implantation they maintained a fairly good development until total absorption was reached. The photomicrographs of figure 1 show the vascularity of the capsule of P. Z. I. and P. Z. I. - cholesterol subcutaneously implanted in guinea pigs and in one human diabetic. It is easy to see the section of blood vessels of different calibres, some of them with a diameter several times greater than a capillary. Furthermore, the injection of the blood vessels with Indian ink offers a clearer picture of the vascularity of the capsule, and it is possible to distinguish two vascular plexus, one *pericapsular*, composed chiefly by arterioles, and another *intracapsular* (fig. 2). It is interesting to note that some intracapsular capillaries reach the inside boundaries almost touching the pellet. In the experiments of more than 30 days, microhemorrhages in contact with the pellet and the adjacent giant cells were found (fig. 1). The study of the calibre, wall and adventicial cells of those blood vessels permit us to state that the intracapsular plexus is formed by arterioles, capillaries and venules, richly anastomosed. Cholesterol and papaverine exhibit in their capsule the same vascular patterns above described. It is important to note that the vascular network is pericapsular and intracapsular not only for the P. Z. I. implantation, but for all substances of slow absorption studied by us (stilbestrol, testosterone, isuprel estearate and Flax's thread; Silva (1951).

b) *Absorption of P. Z. I. implanted subcutaneously.*

Daily absorption estimated by the decrease in weight of the pellets. — In previous studies we observed a rate of absorption for P. Z. I. - cholesterol implanted pellets, of aproximately 1 % daily (Vargas and Koref, 1949). Lewin (1949) confirmed this percentage for the first 40 days, noticing a reduction of 0.5 % in the subsequent 50 days. However, for the pure P. Z. I. pellets, he observed a daily rate of absorption of 1.3 %

in the first 90 days and of 0.3 % thereafter. Curiously enough, with P. Z. I. alone the total absorption took place around 230 days instead of 130 days for P. Z. I. - cholesterol mixture.

TABLE II

Microscopic observations on the frequency and development of the blood vassels of the capsule of P. Z. I. - cholesterol

Substance	Nº of impl.	Days of implant.	Vessels * neoform.	Hyper-hemiae	Hemosi-derine
Cholesterol	3	5 - 8	++	+++	0
	6	16 - 40	++	+++	+
	4	45 - 60	++	+++	++
	8	94 - 130	++	++	0 to +
Protamine-	5	1 - 2	0	++	0
zinc-insulin	7	5 - 17	+++	++	0
	3	30 - 45	++	+++	+
Protamine-zinc-insulin	1	62	+++	+	0
+ 10 % choles-terol	1	204	+++	+	0
Protamine-	15	1 - 5	0	++++	0
zinc-insulin	15	8 - 22	+++	++	0 to +
+ 50 % choles-	7	32 - 51	+++	++	0 to +
terol	5	60 - 118	+++	++	+

* The vessels of neoformation refer to inner vascularity or intracapsular vessels; hyperhemiae, hemosiderine, include the pericapsular blood vessels.

In 75 subcutaneous implantations of P. Z. I. - cholesterol, included in Table I, we confirmed the above observations on the decrease in weight of the pellets, with an average of 1 % daily.

Toxic and hypoclycemic effect upon rabbit and guinea pig. — If insulin were absorbed during implantation, it would produce fatal coma by hypoglycemiae every time the dose reaches a toxic level. We have studied the frequency of fatal hypoglycemic coma during the first 48 hours after implantation of 51 to 1200 U per kg, in 148 animals. Figure 3 summarizes the observations without considering the type of P. Z. I. implanted in the rabbit or guinea pig. It gives a better inkling of the early mortality, proving the absorption of the insulin implanted.

In a series of 10 guinea pigs implanted subcutaneously with 148 to 298 U per kg of P. Z. I. - cholesterol, the fasting blood sugar content was determined the day following implantation. All but one had hypoglycemiae and two died, having less than 39 mg % of blood sugar level.

Death within 48 hours after implantation is frequent for P. Z. I.; it is exceptional for other substances used here. Thus, in 21 implantations of cholesterol and 57 of papaverine no death was observed. We have included in this control group a prolonged period of observation (up to 130 days), because some animals implanted with P. Z. I. died later around the 3rd. and 10th. day of implantation.

The implantation of Neutral P. Z. I. - cholesterol, in doses of 184 to 935 U per kg in 20 rabbits, produced severe shock and for the first time, spontaneous fracture of the femur. Gilliland and Martin (1951) reported that implantations of 10000 U (about 5000 U per kg) of P. Z. I.-cholesterol, did not produce hypoglycemic effects on rabbits. They saw cases of fatal hypoglycemic shocks with implantations of Neutral P. Z. I.

Glycemiae-glycosuria control in human diabetics. — The regular absorption of insulin implanted is specially well demostrated by the experiments in human diabetic patients. We have employed one dose per implantation equivalent to 100 times the daily dose of P. Z. I. injected. Thus, of 49 implantations in 29 diabetic patients, 43 were able to replace the injections; 27 of these 43 implantations satisfactorily regulated the glycemiae-glycosuria for a period of 20-81 days (63 %), and 9, only for 15-20 days. In three patients there was a lack of insulinic activity and in 4 instances the follow up was insufficient. Finally, in 5 implantations we were able to diminish the daily dose of P. Z. I. according to our calculations (Vargas, 1949). Figure 4 illustrates some of the results obtained in the control of the blood sugar level of diabetic patients implanted with P. Z. I.-cholesterol.

c) *Important factors in the preparation of P. Z. I. - cholesterol pellets that could influence its absorption.*

The preparation of P. Z. I. - cholesterol pellets is extremely ticklish and several details that at first glance were not apparent, were found to be of importance.

Kind and quality of the P. Z. I. — Apart from its hog or bovine origin, insulin may have other variations according to its manufacture. We have in mind, for instance, the result obtained with P. Z. I. Choay of French origin. This insulin gave excellent clinical result when administered as a suspension. The biological activity in rabbits agreed with that indicated on the label. But when we tried to use it in the preparation of pellets, we found out that the desiccation of the precipitate was possible

only under low temperature. The pellets thus prepared were very rapidly absorbed and disintegrated when they were removed.

Use of alcohol before drying the product. — Gilliland and Martin (1951) introduced a modification in the preparation of P. Z. I. with calcium carbonate: the powder was passed through alcohol and acetone. We

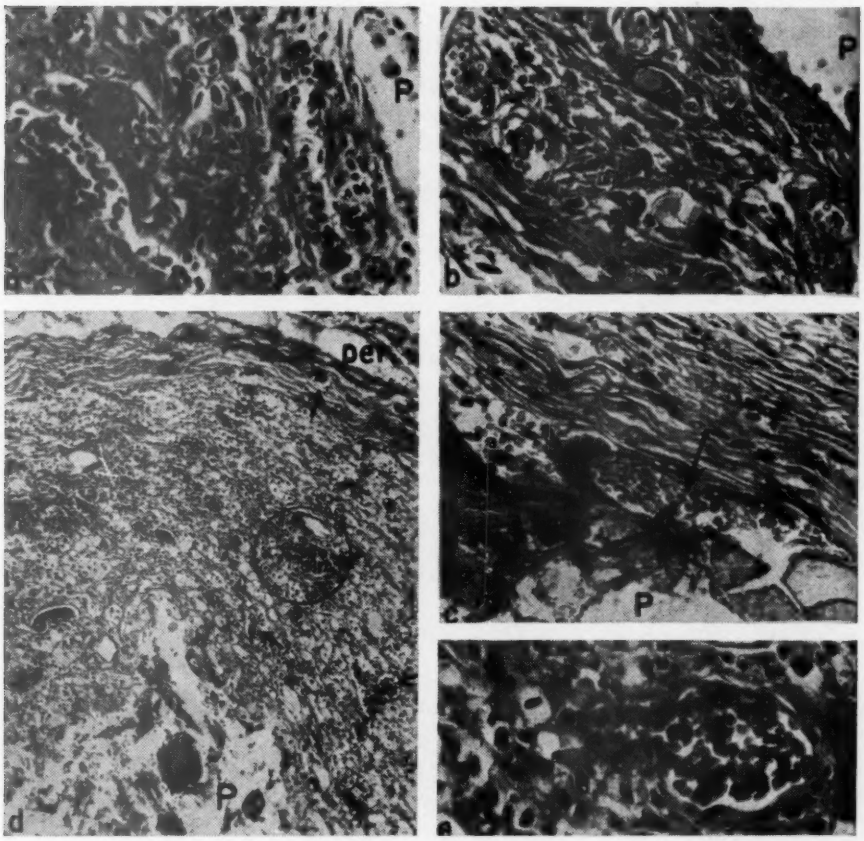

FIG. 1. — *Vascularity of the capsule of P. Z. I. in guinea pigs and in human diabetics. Note the abundance of blood vessels and their distribution near the pellet. P = side of the pellet; per = pericapsular vessels.*
Photomicrographs of histologic samples from subcutaneous implantations: a) guinea pig: P. Z. I., 45 days; b) guinea pig: cholesterol, 18 days; c) guinea pig: P. Z. I. 50 % cholesterol, 51 days; arrows show microhemorrhages surrounding giant cells; d) human diabetic: P. Z. I. 50 % cholesterol, 65 days, general view of the capsule. Pericapsular vessels are shown by arrows at the top. Arrows at the botton indicate intracapsular vessels; e) higher magnification of the zone indicated by the circle in d) showing an arteriole.

prepared a sample of P. Z. I. using alcohol but not acetone. We observed with this material that the pellets recovered after implantation in guinea pigs has a decreased consistency. Besides, the insulin activity was diminished in two biological assays on rabbits and showed a low insulinic effect in four clinical trials. As a matter of fact, 3 of the failures mentioned on page 14 belong to this type of P. Z. I. preparation.

Mixed cholesterol and passage through ether. — We pass P. Z. I. and cholesterol together and in the *same* mortar for two reasons: to dissolve cholesterol and facilitate the mixture with P. Z. I. and to assure a final sterilization. It was possible, however, that ether could have an influence in obtaining the mixture because it is rare that from two substances of very slow absorption there should result one of a more rapid absorption. Moreover, it is unusual for cholesterol to hasten the absorption of P. Z. I. instead of delaying it, as occurs with the steroid hormones. We thought that without using ether it would be possible to get a different mixture with no absorption. In this case, in addition to the very prolonged absorption of P. Z. I. we would have the extremely low absorption of cholesterol. To check this, we treated cholesterol and P. Z. I. with and without ether in *separate* recipients. These experiments showed that ether plays no role in the production of an absorbable compound.

Sterilization of the pellets. — The powder of P. Z. I. aseptically prepared has been sterilized by a treatment with ether. Samples of pellets so prepared were bacteriologically controlled, giving no growth of microbes.

The pellets of P. Z. I. must be sterile because the tissue reaction and the absorption of the implant can be modified by an infection. In a series of 10 implantations in guinea pigs, with non sterile pellets, all the capsules developed infection, showing a macroscopic piogenic inflammatory reaction. The capsules in these cases had intense hyperhemiae with puriform material, composed chiefly by polymorphonuclears ("piogenic reaction"). Exceptionally it is observed a true supuration with abcess formation. In cases without infection, the capsule was less hyperhemic, with scanty exudate, integrated by histiocytes and plasmatocytes without polymorphonuclears ("histiofibroplastic reaction"). In the first instance, the pellet has a tendency to exhibit less consistency and a greater decrease in weight. On the contrary, in the histiofibroplastic reaction the pellet kept its shape and consistency, and the diminution of weight corresponds approximately to 1 % daily, as it was previously mentioned.

Technique of the preparation of the P. Z. I. - cholesterol pellets. — We conclude, therefore, that under present conditions it is not advisable to introduce modifications in the preparation of the P. Z. I. pellets. For this reason we here give a plan to prepare the pellets.

1) *General observations.* — It is necessary to work in aseptic conditions. The glass material must be sterilized and the machines or instruments, treated with direct flame.

2) *P. Z. I. powder preparation.* — If commercial P. Z. I. is used, the following steps are necessary: a) centrifugation; b) decantation and c) desiccation. Centrifugation at a rotor speed of 3000 rpm during 15 minutes. Desiccation during 3 days in a vacuum over sulphuric acid until a dark brownish coloration is obtained (Neutral P. Z. I. remains white).

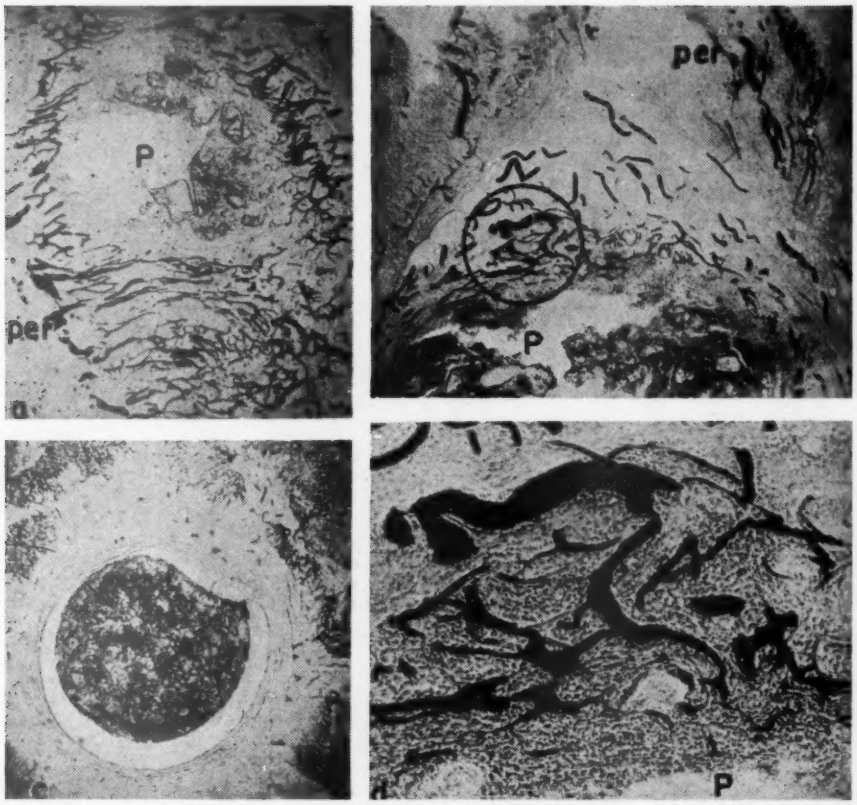

FIG. 2. — *Blood vessels of the capsule of P. Z. I. injected with Indian ink in guinea pigs. In a), b), and d), photomicrographs of histologic preparations from subcutaneous implantations of P. Z. I. 10 % aluminum monoestearate, 30 days; in b) the circle shows intracapsular vessels; in c) intrahepatic implantation of P. Z. I. 50 % cholesterol, 10 days, showing fibrotic encapsulation and absence of blood vessels with intact pellet. P: pellet; per: pericapsular vessels.*

Weigh aseptically and calculate U per mg, starting from the original value.

3) *Mixture with cholesterol.* — To equal quantity of P. Z. I. powder add chemically pure cholesterol (sterilized at 98° C) in a sterile mortar. Mix the substances and pour on ether till it covers the P. Z. I. powder and cholesterol completely. Cover with a perforated sterilized paper to facilitate evaporation of the ether. The next day, after ether evaporation, mix again carefully in the same mortar.

4) *Compression of the pellets.* — We recommend the direct flame

before and during the compression. Operator must wear a mask. Avoid excessive pressure.

Discussion

From our experiments we have been able to appreciate the following points: 1) decrease in weight of the implanted pellets at a rate of approxi-

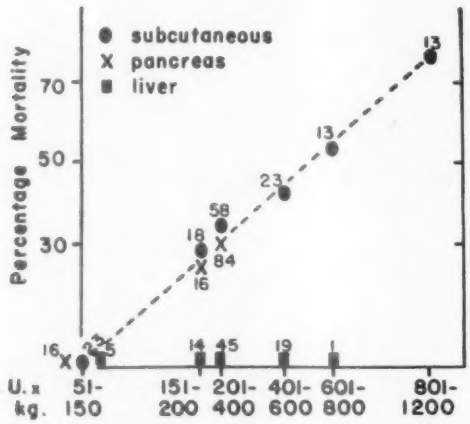

Fig. 3. — *Mortality during the first 48 hours post-implantation. Data of rabbits and guinea pigs, implanted with P.Z.I. or P.Z.I. cholesterol. Abscissae, I. U. of insulin per kilo, in logarithmic scale. The figures in the vicinity of each point indicate the number of animals implanted with the respective dose.*

mately 1 % daily for P. Z. I. - cholesterol 50 %; 2) vascularity of the capsule; 3) hypoglycemic shock and fatal coma after implantation of toxic doses (54 % of mortality during the first 48 hours post-implantation, with 601-800 U per kg); 4) control of the hyperglycemiae, and glycosuria in the human diabetics, without the help of the insulin injection, during a period of 20-81 days. Points 1, 3 and 4 prove an absorption of the P. Z. I. implanted. Moreover, in 2 animals we observed fatal hypoglicemiae on the 6th. and 22nd. days after implantation, on account of prolonged fasting. The first case was a female guinea pig weighing 310 g, given 258 U per kg. It suffered such an intense shock that the blood sugar content fell to 6 mg %. The second case, a male rabbit of 2400 g, was able to withstand a dose of 416 U per kg perfectly well. On the 22nd day, after 12 hours fasting, it was found dead with signs of intense convulsions.

The experiments of Gilliland and Martin (1951) in normal and diabetic rabbits, do not agree with these observations of P. Z. I. - cholesterol. In order to find an explanation for these contradictory results we have thought that, for some unknown reason, a change in the physical

properties of the P. Z. I. has occured during the manufacture of their pellets. This would give a compound which would behave differently in the subcutaneous tissues, producing encapsulation of the implant with

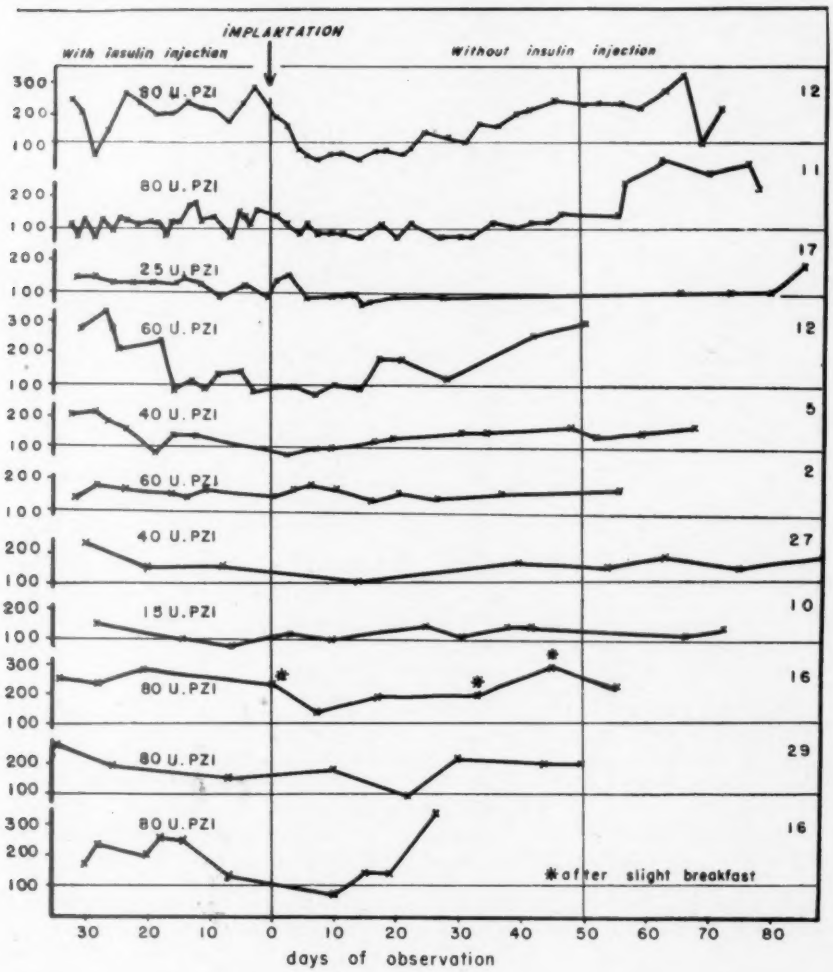

FIG. 4. — *Glycemiae before and after insulin implantation in diabetic patients. Ordinates: glucose g %. The end of the curve corresponds to the moment the insulin injections were resumed. The figures of insulin units indicate the daily insulin requirement before the implantation. The patient's numbers are indicated on the rigth side of the graph. Cases 11 and 12 from Vargas, Lewin and Winter (1950).*

absence of blood vessels and blockade of the absorption. This is in accordance with our observation on intrahepatic implantation of P. Z. I., where a similar encapsulation was noted with an unusual impediment of the absorption (Vargas and Maturana, 1953).

Baisset, Lichwitz and Montastruc (1953), using a similar compound to ours, observed a decrease in the weight of the pellets of P. Z. I. -cholesterol, a vascular capsule and an insulin effect equivalent to the dose calculated. These experiments were made in dogs rendered diabetic by pancreatectomy or by administration of alloxan. The P. Z. I. implanted was sufficient to replace the insulin injections for as long as 2 to 3.5 months. The fasting blood sugar levels of these animals were 80-100 mg %.

In regard to the vascularity of the capsule, it is important to note that it is peri-capsular as well as *intracapsular*. We have not found previous data on these intracapsular vessels. This vascularity has a general value for all non irritating substances insoluble in water and with a prolonged rate of absorption. On the contrary, with the scarcely hydrosoluble substances such as phenobarbital and procaine-penicillin, where the absorption takes place in five days, no blood vessels were encountered (Silva, 1951). All these observations agree with the concept that an aqueous insoluble substance requires, for its absorption, the existence of blood vessels in the capsule. They would develop a network which corresponds to the surface for dialysis.

SUMMARY

The absorption of P. Z. I. - cholesterol pellets, implanted subcutaneously, has been corroborated by: a) decrease in weight of the pellets with a daily diminution rate of approximately 1 %; b) fatal hypoglycemiae when the implanted dose is toxic (over 150 U per kg); c) control of the diabetic state of human patients by subcutaneous implantations of P. Z. I. - cholesterol.

The histologic study of 80 specimens from subcutaneous implantations of P. Z. I., P. Z. I. - cholesterol and cholesterol, definitely demonstrate the presence of blood vessels in the capsule formed around these substances. There is a good rate of vascular development from the fifth day of implantation, keeping this state until total absorption is accomplished. The vascularity of the capsule is characterized by a *pericapsular* plexus, composed of arterioles. These vesels penetrate the capsule and build a rich plexus of anastomosed capillaries and venules (*intracapsular vessels*). These vessels just reach the boundaries of the pellet. The presence of blood vessels in the capsule seems to be indispensable in order to obtain a regular P. Z. I. absorption.

We analysed some details in the preparations of P. Z. I. - cholesterol pellets, which might be responsible for obtaining an inadequate material for implantation.

We are indebted to Professor Dr. Juan Vial, of the Department of Anatomy, for his collaboration in the experiments with Indian ink.

The P. Z. I. was generously supplied by E. R. Squibb and Sons (U. S. A.) and Laboratories Pel. (Chile); the Neutral P. Z. I., by the Connaught Medical Laboratories (Canada), through the courtesy of Dr. C. H. Best.

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RELATIONSHIP BETWEEN FOLIC ACID AND SEXUAL HORMONES IN TOADS AND RATS

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HERTZ OBSERVED in 1944-45 that in chickens fed a diet poor in folic acid the response of the oviduct to stilbestrol was markedly depressed and that feeding the vitamin restored the response to normal. Goldsmith et al (1948-50) observed that the response of the oviduct of *Rana clamitans* to estradiol benzoate was markedly reinforced when the batrachian was treated with folic acid. Hertz (1948) observed in female monkeys that the diet poor in folic acid inhibited the normal response by the genitalia to estrogens. Addition of the vitamin to the diet restored this response to normal.

In prepuberal rats (Hertz and Turner, 1944) the growth of the uterus provoked by estrogens was inhibited by antagonists of folic acid. The inhibition was reversed when folic acid was given.

The above mentioned works led us to investigate the reactions in the toad usually employed in our laboratory (*Bufo arenarum* Hensel), both prepuberal and adult, males and females, when treated with estrogens or androgens with or without folic acid. A similar study was also made in rats.

MATERIAL AND METHODS

A) Toads: Sexually immature females of 40 to 85 g of body weight were divided in 4 groups of 10 animals each: 1) uninjected controls; 2) injected 3 times a week with 0.2 mg of folic acid into the dorsal lymphatic sac; 3) injected 3 times a week with 0.1 mg of estradiol benzoate into the dorsal lymphatic sac, and; 4) injected with folic acid and estradiol simultaneously with the same doses and intervals employed in the other groups. At the beginning of the experiment, the oviduct of one side was removed from each animal and weighed. After 3 weeks of treatment, the toads were sacrificed and the remaining oviduct of each was removed and weighed. As these animals were in the growth period, they were fed calf fresh liver 3 times a week so as to avoid alterations in the experiment caused by prolonged fasting.

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In another experiment, 50 adult female toads weighing from 100 to 150 g were forced-fed for one month with calf fresh liver in order that all the animals would be in the same nutritional state. Ovulation was induced in these animals by injection of *pars distalis* of hypophysis of the toad, in order to exhaust the oviduct, which would, in turn, facilitate the study of uterine growth. All the animals were then laparotomized and the oviduct of one side removed. They were divided into 5 groups of 10 toads each: 1) controls under fasting; 2) fed controls (to observe whether feding can accelerate growing); 3) fed injected with folic acid; 4) fed injected with estradiol benzoate; and; 5) fed injected with both substances. Doses and intervals were similar to those employed for the small animals.

One hundred male toads, sexually immature and weighing from 10 to 30 g were divided into groups of 20 toads each; 1) controls, sacrificed on the first day of the experiment; 2) controls sacrificed at the end of the experiment; 3) injected 3 times a wek with 1 mg of folic acid into the dorsal lymphatic sac; 4) injected twice a week, with 1 mg of testosterone propionate into the dorsal lymphatic sac, and; 5) injected with folic acid and testosterone propionate under the same doses and intervals indicated for groups 3 and 4. The injections were given for a period of 3 weeks.

B) Rats: Castrated female rats*, 21 days of age and weighing 35 to 45 g were divided in 4 groups of 10 animals and were treated for 10 days: 1) controls, 2) injected daily, subcutaneously, 1 μg of estradiol benzoate; 3) injected daily, subcutaneously, 3 mg of folic acid, and, 4) injected with estradiol benzoate and folic acid with same doses and intervals as used for lots 2 and 3.

Sixty male rats, 21 days of age, and weighing between 30 and 40 g, were divided into 4 groups of 15 rats each and were treated for 15 days as follows: 1) uninjected controls; 2) injected subcutaneously with folic acid, 1 mg every other day; 3) injected subcutaneously 250 μg of testosterone propionate, every other day, and, 4) injected with folic acid in the same way as groups 2 and 3.

Uterus, testicles, coagulant glands and seminal vesicles were weighed when the animals were sacrificed. For histological study, the organs were fixed in Helly, imbedded in paraffin and sections stained with hematoxylin-eosin and periodic acid-Schiff method (McManus).

RESULTS

A) The results obtained in female toads are presented in table I. The synergistic effect of folic acid with estradiol is clearly evident in the prepuberal toads. Estradiol given alone also produced a weight increase but it was not as large. Folic acid given alone had no effect on the oviduct weight in sexually immature toads.

In uninjected adult female toads, both fasted and fed, and in those injected with folic acid, there was a loss of weight in the oviduct.

* These rats were fed the common laboratory diet, as follows: wheat, 8 %; corn, 8 %; dry bread, 20 %; and milk, 64 %; alfalfa was also fed once a week.

TABLE I

Female toads, sexually immature and adult, fed, treated with folic acid and/or estradiol benzoate, for 3 weeks. Each figure represents the mean average of 10 toads.

Group	Prepuberal toads				Adults toads			
	Body weight g	Oviduct initial weight g	Final weight g	Weight gain (% of initial weight)	Body weight g	Oviduct initial weight g	Final weight g	Weight gain (% of initial weight)
Controls	60	0.067 ± 0.010 (1)	0.069 ± 0.015	3.5	127	1.036 ± 0.126	0.848 ± 0.107	— 18.14
Folic acid (.02 mg/3 times a week)	61	0.070 ± 0.011	0.072 ± 0.017	3.1	129	1.059 ± 0.230	0.126 ± 0.177	— 3.11
Estradiol benzoate (0.1 mg 3 times a week)	61	0.083 ± 0.009	0.241 ± 0.052	190.0	129	1.141 ± 0.110	1.198 ± 0.203	5.04
Folic acid + estradiol benzoate (dosage same as above)	61	0.059 ± 0.013	0.739 ± 0.102	1152.0	130	0.943 ± 0.141	1.160 ± 0.097	22.98
Fasted controls	—	—	—	—	134	1.333 ± 0.103	1.020 ± 0.099	— 23.48

(1) Mean ± Standard Error.

In those injected with estradiol a slight increase of weight was observed. Administration of both substances produced a much larger weight gain. The histological study was carried out by Dr. A. F. Cardeza: a) in toads treated with folic acid, the glands of the oviduct showed very little development, with only few grains of intracellular mucin; the papillary crests were also poorly developed having epithelium that was flat and free of ciliae (fig. 1, a and c); b) in toads treated with estradiol, the picture was similar to the previous one but the amount of mucin present was greater; c) in toads treated with both folic acid and estrogen there was a much more marked glandular development than in the two previous ones; the cells of the fundus of the glandular sac contained abundant grains of mucin; the glandular tubes were greatly distended with mucin secretion. The epithelium of the papillary crests were tall, cylindrical and had many ciliae (fig. 1, b and d).

The results obtained in sexually immature male toads, are presented in table II. The final body weight in all groups was less than that at the beginning. The weight of the testicles was only increased in toads injected with testosterone propionate, both with or without folic acid. The latter potentiated, in a significant way, the action of the male hormone. The pigmentated callosity on the first finger of the male toad, which is a secondary sexual character, appeared only under the action of testosterone and its frequency was increased by folic acid.

B) The results obtained in prepuberal castrated female rats are presented in table III. No significant increase in body weight was seen in the rats as the result of treatment. The weight of the uterus was increased significantly in rats given estradiol benzoate, alone or with folic acid, being more evident in the latter case. Folic acid alone had no effect on the uterine weight.

The results obtained in the experiments in prepuberal male rats are presented in table IV. There was no effect of treatment on body weight. The testicular weight in animals injected with testosterone was less than that in controls. The testicular weight was even smaller in those treated with both substances.

The weight of coagulant glands and seminal vesicles was markedly increased in the rats injected with testosterone, either alone or with folic acid, although the increase was greater when injected with both substances. Folic acid alone had no effect on the organs.

DISCUSSION

The fact that folic acid reinforces the action of estrogens is a phenomenon most clearly seen in prepuberal toads in the oviduct. Folic acid given alone had no appreciable effect on the oviduct of the prepuberal toad. When estrogen alone was given, there was a definite increase in the weight of the oviduct with very small changes in the histological picture. Following the administration of both estrogen and folic acid, there was a most pronounced gain in weight of the oviduct which was accompanied by marked development of the glandular cells and accumulation of secretion in both the glandular tubes and the lumen of the oviduct.

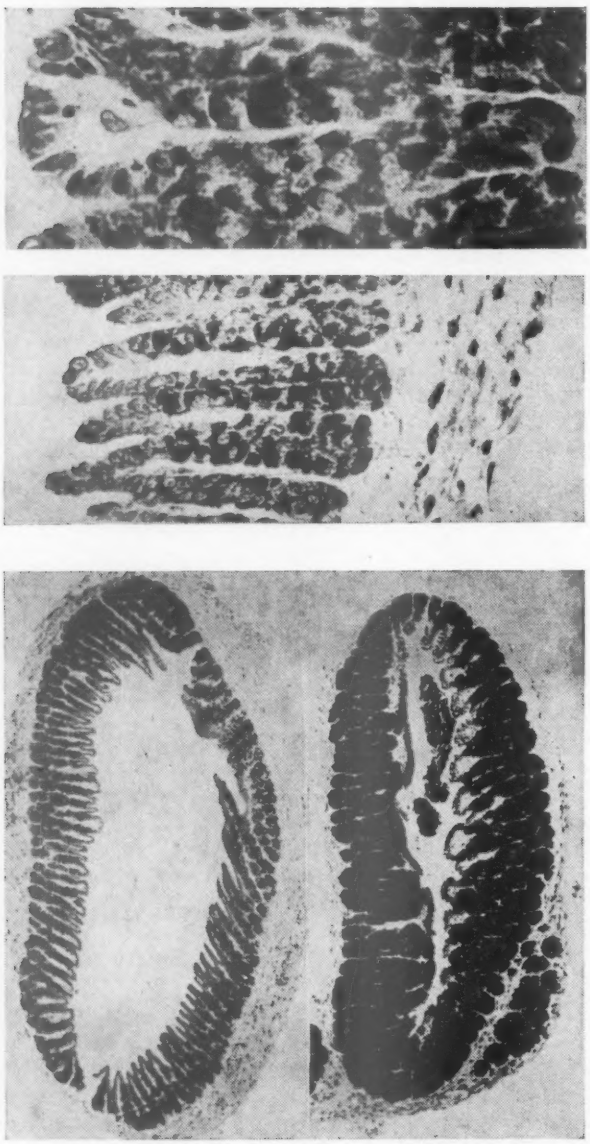

FIG. 1. — *Transverse section of the oviduct of sexually immature toad.*
a) & c) *treated with folic acid.*
b) & d) *treated with folic acid and estradiol benzoate.*
Magnification: a & b) × 80; c & d) × 480.

TABLE II

Male toads, sexually immature, weighing 10 to 30 g, treated with folic acid and/or testosterone proprionate, for 3 weeks. Each figure represents the mean average of 20 toads.

Group	Body weight g		Weight of testes mg		Number of toads with dark thumb callosity
	initial	final	initial	final	
Initial controls	16.9	—	10.1	—	0/20
Final controls	17.3	15.6	—	10.5 ± 2.1(1)	0/20
Folic acid (1 mg, 3 times a wek)	16.8	15.7	—	9.9 ± 1.4	0/20
Testosterone propionate (1 mg, twice a week)	19.7	18.2	—	21.0 ± 3.3	9/20
Folic acid + testosterone prop.	18.3	17.2	—	27.7 ± 3.8	15/20

(1) Mean ± Standard Error.

Reinforcement of the stimulating effect of estradiol on the oviduct of the amphibian (Houssay, 1950; Galli-Mainini, 1950; Penhos, 1950) has been observed also by Goldsmith and coll. (1948-1950). The latter attributed this synergistic action to an acceleration of nucleic acid formation and cellular division.

A similar consideration could be applied to the testosterone propionate which, under the doses employed, is potentiated by folic acid,

increasing the weight of the testicles and accelerating the apparition of the thumb callosity.

The lack of increase in body weight was due to the insufficient

TABLE III

Prepuberal castrated female rats 21 days of age, injected for 10 days. Each figure represents the mean average of 10 rats.

Group	Body weight g		Uterus weight	
	initial	final	mg/100 g	Difference % of Control
Controls	39.8	66.4	27.5 ± 4.6(1)	—
Estradiol benzoate (0.1 mg/day)	39.2	67.4	84.7 ± 5.3	+ 208
Folic acid (3 mg/day)	40.0	66.4	24.4 ± 6.2	— 11.3
Estradiol benzoate + Folic acid	39.1	66.7	98.8 ± 6.9	+ 259.3

(1) Mean ± Standard Error.

amount of food given to the animals. The calf fresh liver in larger amount could exert an effect similar to the folic acid.

In castrated female rats, the well known effect of estradiol benzoate on the growth of the uterus (Lauson and coll., 1939) was potentiated by the folic acid, which when given alone could not prevent the atrophy of the organ following castration.

TABLE IV

Prepuberal male rats, 21 days of ate, treated with folic acid and/or testosterone propionate, for 15 days. Each figure represents the mean average of 15 rats.

Group	Initial body weight g	Final body weight g	Weight of testes mg	Weight of coagulating glands and seminal vesicles mg
Controls	34.6	70.5	613.2 ± 61.3 (1)	18.9 ± 3.7
Folic acid (1 mg/every other day)	33.0	75.9	634.7 ± 77.3	17.5 ± 4.2
Testosterone propionate (250 ug/every other day)	35.5	74.3	442.1 ± 36.6	82.9 ± 7.8
Folic acid + testosterone propionate (above dosages)	35.3	73.1	304.3 ± 33.8	112.6 ± 10.3

(1) Mean ± Standard Error.

The direct effect of the testosterone propionate on the growth of secondary sexual organs of prepuberal male rats and the indirect, inhibitory effect, exerted though the hypophysis, on the growth of the testicle, were potentiated by folic acid. On the contrary inhibition of testicular growth was not observed in toads, under the doses and time employed.

SUMMARY AND CONCLUSIONS

1) In toads, folic acid potentiated both the effect of estradiol on the growth of the oviduct and of the testosterone propionate on the increase of weight of the testicles and apparition of thumb callosity. These effects were much more evident in prepuberal toads.

2) In prepuberal rats, folic acid reinforced the response of the uterus to estradiol benzoate and the growth of coagulant glands and seminal vesicles by the action of testosterone propionate.

3) Folic acid had no effect on sexual or annexal glands of rats and toads.

We wish to express our appreciation to Lederle Laboratories for the supply of folic acid (Lederfolic) and to Química Schering for the supply of Testosterone Propionate (Testoviron).

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ASPECTS OF HEMODYNAMICS OF THE CAROTID SINUS IN MAMMALS

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MUCH WORK has been done on physiology of the carotid sinus; a resumé of the classical data may be found in the monograph of Heymans, Bouckaert and Regniers (1933).

The more important recent contributions on sinus activity appear to date from Palme's investigations (1944).

The new findings concern: 1) The mechanism of excitation of chemoreceptors and pressoreceptors. 2) The influence of the state of contraction or relaxation of the sinus wall on the excitation of the local baroreceptors. 3) Possible sympathetic-adrenergic control of the sinus wall. 4) The sinusal hemodynamics:specially the study has a bearing on treatment on account of the operation of ligation of the carotid in cases of high carotid aneurisms.

In this paper we shall describe recent investigations on the influence of the sinus wall on the stimulation of its baroceptors and a summary of some aspects of sinual hemodynamics.

A) *State of the sinus wall and excitation of pressoreceptors.* — Numerous factors tend to prove in agreement with Heymans et al. (1950) that contraction of the smooth muscle of the carotid bifurcation stimulates the local baroceptors; this effect provokes systemic reflex hipotension. Adrenaline, arterenol and pitressin applied locally on the sinual wall operate by this mechanisms (Mazzella, Wang, Heymans and De Vleeschhouwer, 1952), and not by direct excitation of sinual receptors as Palme claimed (1944) (Fig. 1).

In confirmation of the facts above noted it is showed (Landgren, Neil and Zotterman, 1952) that adrenaline, arterenol and pitressin on contracting the sinus, excite its baroceptors as is evidenced by the increase of the discharge of small baroceptor spikes. Also after local application

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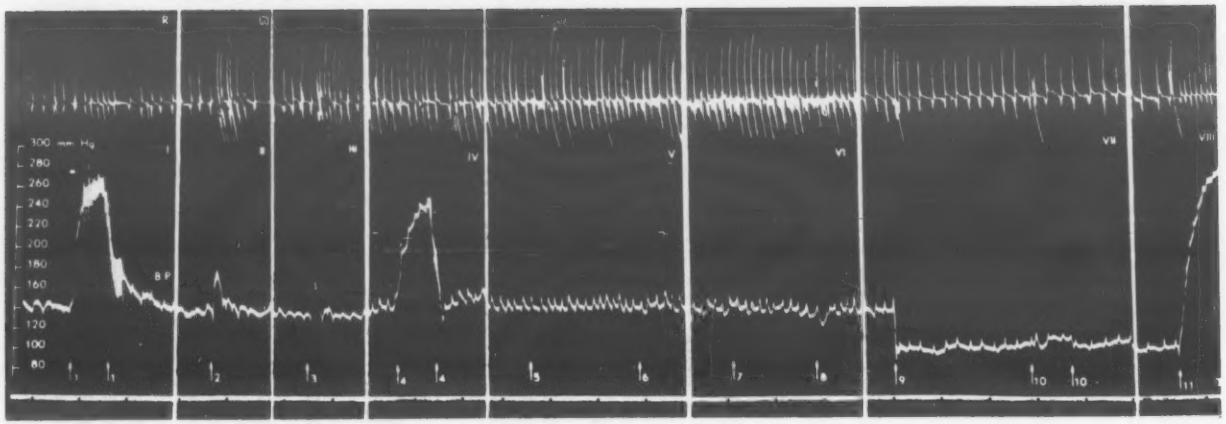

FIG. — 1. — *Chloralosed dog. Bilateral vagotomy.*

Records: B P-femoral arterial pressure with mercurial manometer (in all figures) R-respiration, with neumograph. Time: 3 seconds.

↑ 1 ↑ 1: *Clamping and unclamping of common carotids.*

↑ 2: *Injection of 1mg of KCN into the left common carotid. Respiratory and hypertensive reflexes.*

↑ 3: *Injection of 0,1 mg of Lobeline-HCl into the same artery, provokes identical reflexes as in 2.*

Between III and IV, injection of acetic acid 0,5 N, 5 cc. in the right common carotid and 8 cc. in the left carotid.

↑ 4 ↑ 4: *Clamping and unclamping of common carotids. Reflex hypertension.*

↑ 5: *Injection of 1 mg of KCN, into the left common carotid, is not followed by respiratory reflex.*

↑ 6: *Injection of 0,1 mg of Lobeline into the same artery does not elicit reflexes.*

↑ 7 ↑ 8: *Injections of KCN and Lobeline, respectively, into the right common carotid, do not provoke reflex reactions.*

↑ 9: *Local application of 0,2 cc. 1 ‰ of noradrenaline (bitartrate) on each carotid sinus.*

↑ 10↑ 10: *Clamping and unclamping of common carotids. There is no hypertensive reflex.*

↑ 11: *Complete sinual denervation of Hering's nerves. Systemic hypertension. (Mazzella, Wang, Heymans, et De Vleeschhouwer, 15).*

This experiment shows that after blocking the carotid chemo ceptors with acetic acid, local injection of noradrenaline on the carotid sinus still provokes reflex systemic hypertension.

of adrenaline, the baroreceptors producing large spikes seems to be more reactive to changes of sinual presure (Landgren, 1952). The adrenolytic agents dibenamine and ergotamine, applied to the carotid wall prevent the local constricting action of adrenaline and noradrenaline (Heymans, De Vleeschhouwer and Van den Heuvel-Heymans, 1951).

Ephedrine and neosynephrine (sympatol), sympathomimetic substances, also produce stimulation of the carotid pressoceptors (Heymans and Mazzella, 1952).

On the other hand there are substances which relax the sinual musculature; sodium nitrite is one of these.

Papaverin and priscol injected in the sinual adventitia of the dog anesthetize the baroceptors and produce a corresponding arterial hypertension by blocking the sinual nerve (Landgren, Neil and Zotterman, 1952).

Landgren (1952) has made an important experimental analysis of the sinual distensibility by registering the relation pressure-volume of the isolated sinus of the cat. He showed that adrenalin permits a decreased distension of sinual wall by pressures of less than 100 mm Hg, but an increased distension betwen 100 to 200 mm. Sodium nitrite modifies the parietal distensibility rather in the opposite direction from that of adrenaline. These studies emphasized that during sinual contraction there is an increased distensibility of the carotid sinus region.

For the most satisfactory interpretation of the reactivity of the carotid sinus we think necessary draw attention to the following hemodynamics factors (Burton, 1951): 1) the vascular *active tension* caused by the contraction of sinual muscle, which normally depends of the local blood pressure and sympathetic mechanism; 2) the *total tension* of sinual wall which is a function of the blood pressure and the radius of the sinual curvature, and is expressed in Laplace's equation: $T = PR$.

Sinual contraction provoked by adrenaline increases active tension but R and T are decreased. Sodium nitrite, on the contrary, dilates the carotid sinus, the active tension is decreased, R and T are increased (Landgren, 1952).

Distension of carotid sinus (by blood pressure) increases T, but causes by reflex a decrease of systemic presure. With these and related experiments we believe that the effect of the constricted sinus on its distensibility should be important in stress. In fact we may consider the carotid sinus of dogs as a very small reservoir; by action of adrenaline or sympathoadrenergic stimulation the distensibility of the pouch would increase so that its function as an elastic reservoir for blood supply to the brain and for reflex homeostasis of arterial pressure, would be augmented.

In addition to the findings above summarized, in a recent paper De Vleeschhouwer (1954) maintains that noradrenaline injected into the wall of the aortic arch determines systemic reflex hipotension, probably due to the contraction of the vascular muscle.

If this result is confirmed it should be possible to extend to the aortic nerve the studies made on the sinus nerve, including the role of the aortic vasomotor tonus on reflex homeostasis of blood pressure.

B) *Cervical superior ganglion and carotid sinus.* — First Palme (1944) and then Kezdi (1954) observed that excitation of the postganglionic fibers of the C. S. G. produces a reflex decrease of arterial pressure, an effect probably due to the sinual contraction. We have observed (unpublished experiments) that this action is obtained only with a long electrical stimulation.

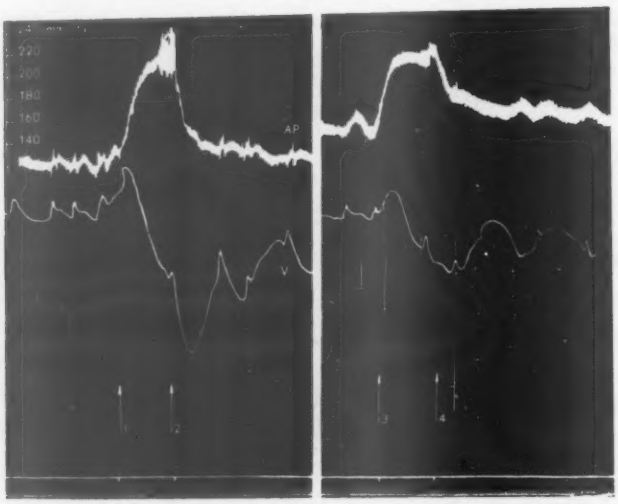

FIG. 2. — *Chloralosed dog. Bilateral vagotomy. AP-femoral arterial pressure. V-splenic plethysmogram.*
Time: 3 seconds. 1-2 clamping and unclamping of common carotids arteries; 3-4, external carotids previously occluded, clamping and unclamping of common carotid arteries. Note the reduced splenic and hypertensive variations. (Mazzella and Migliaro, 14).

Besides, injection of noradrenaline into the adventitia of the carotid sinus sensibilized by sympathetic denervation were made in dogs (Mazzella, preliminary experiments, unpublised). In this condition a reflex hypotension is produced which is greater than that produced on the innervated sinus. Therefore, the initial state of relaxation or contraction of the sinual wall, depending on the sympatho-adrenergic mechanism, affects the stimulation of the Hering's nerve, one of the points of origin of the reflex regulation of the arterial pressure.

The above facts put emphasis on the wall of the carotid sinus, and probably also on the aortic wall for the production of reflex modifications of arterial pressure and cardiac frequency. In the following sections other aspects of some of the preceding observations are discussed.

C) *Sinual hemodynamics.* — Investigations of Wang, Mazzella and Heymans (1952) are concerned with the afflux of blood to the sinual

zone when the common carotids of the dog are ligated. In this case the blood arrives by the occipital coming from the vertebrals; in some cases it may arrive also from the brain by the internal carotid. The occlusion of the vertebrals increases the sinual hypotension. The ligature of one of the common carotids reduces the pressure in the opposite sinus by way of the external carotid; a greater reduction of sinus pressure is observed

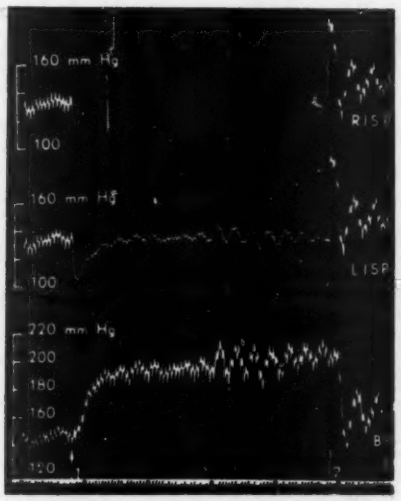

FIG. 3. — *Chloralosed dog, partial curarization and artificial respiration. B P: femoral arterial pressure. ISP: intrasinual pressures. Time: 3 seconds. After occlusion of lingual and external arteries on both sides, clamping of common carotid arteries is made. The result is systemic hypertension, while the intrasinual pressures reached a level slightly higher than the control. (Wang, Mazzella and Heymans, 18).*

when the two common carotids are ligated (Chungcharoen et al., 1952, Mazzella and Migliaro, 1953).

The last named authors have shown, on the other hand, that the occlusion of the external carotid preventing the outflow on the sinual blood and maintaining a high pressure in the sinus, not only limits the reflex hypertension caused by the occlusion of common carotids but also limits tachycardia and the concomitant splenic and renocontraction (fig. 2).

Back flow to the carotid sinus. — In different experiments (Wang, Heymans and Mazzella, 1952, Mazzella and Migliaro, 1953), it is shown that after occlusion of the common carotids and because of the back flow, the sinual pressure may attain a considerable value without modifying the reflex hypertension (figs. 3 and 4).

This finding seems, "a priori", in contradiction with those of Hering, Koch (Heymans, 1933), that any change of intrasinual pressure modifies reflexly the systemic pressure. But these two groups of observations seem less irreconcilable when it is observed that in the pressor reflex intervene: the carotid sinus, vegetative centers (Neil, Redwood and Schweitzer, 1949), and the vascular periphery. Similarly the mechanism of sinual excitation depends on two factors: the blood pressure and the

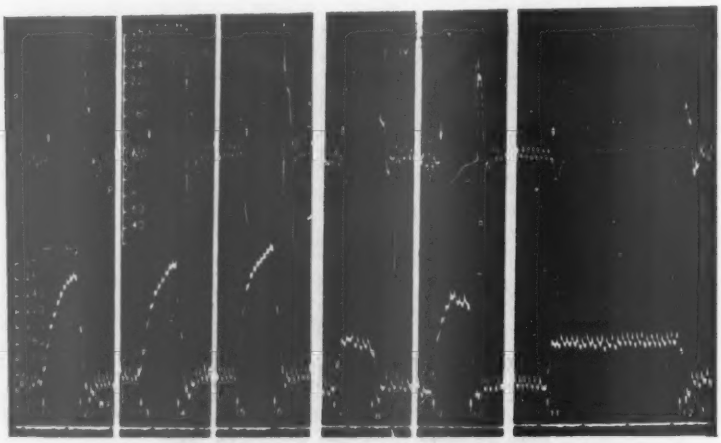

FIG. 4. — *Records made in a chloralosed dog. BP: femoral arterial pressure; ISP: intrasinual pressure. Time: 3 seconds.*

I. *Clamping of both common carotids provokes a falling of sinual pressure and systemic hypertension.*

II. *Both occipital arteries clamped. Slight improved systemic hypertension and decreased sinual back flow.*

III. *Clamps on occipital and internal carotid arteries on both sides. Note the further improved sinus response. The sinual pressure reduction in about the same as II.*

IV. *Lingual, external and internal carotid arteries of both sides clamped. Compare with I. Sinus response greatly diminished. Intrasinual pressure shows a trasient reduction and its recovery is complete about 10 sec. of common carotid occlusion.*

V. *Clamps of the lingual external and internal carotid, and occipital arteries of both sides. Compare with I and IV. The sinus response is greatly improved and there is a relatively more maintained intrasinual pressure reduction as compared with IV. Although the intrasinual pressure reduction is slight when compared with I, the sinus response is almost as good as in I.*

VI. *Repeat IV with prolonged common carotid occlusion. Observe the maintained systemic hypertension despite complete return of the intrasinual pressure to the control level. (Wang, Mazzella, and Heymans, 18).*

state of the muscular wall. A fall of arterial pressure decreases the tension of the wall; this decreased tension reduces the stimulation of the Hering's nerve.

Besides, Hauss, Kreutziger and Asteroth (1949, have proved that

if the distension of the carotid sinus is prevented when there is hypertension therein, no reflex systemic hypotension is produced; but in the preceding section it is stated that the same sinual baroceptors may be excited also by a parietal contraction or that there are other pressoceptors sensitive to sinual contraction. It is logical to suppose that these would be the receptors described in the muscular carotid wall (Palme, 1944). This important question is not yet answered; as Landgren says (1952), new histological studies on the locatization of the receptors of the carotid sinus seem to be necessary.

It should be noted that the study of the pressor reflex when both carotids are occluded shows the apparent inefficacy on the sinual wall of the increase of pressure provoked by backflow. In fact, as Wang et al (1952) first and Mazzella and Migliaro (1953) afterwards have observed, the critical intrasinual pressure must be close to the initial sinual pressure in order to modify the existing reflex. It is possible also that there is a phenomenon of adaptation of sinual receptors (Bronk and Stella, 1934) and perhaps a central adaptation.

On the other hand, it has been found that the backflow is produced by the hypertension of vertebral arteries and that it occurs in two phases: first rapidly as the systemic pressure goes up, and then slowly as that pressure is stabilized (Mazzella and Migliaro, 1953).

The backflow has small pulse as compared with the carotid or the femoral, recalling that an evident action of the wave of pulse on sinual stimulation has been demonstrated (Bronk and Stella, 1934).

All these facts show the complex reactions of carotid clamping. We can consider here that the concept that for each point of sinual pressure there is a corresponding point of systemic pressure is based specially on studies on isolated carotid sinus. This technic is related to only one of the vascular factors above mentioned, the sinual pressure, without taking into account the parietal tension. Probably it would be very useful for the study of the problem to record the potentials of sinus nerve at the same time with the sinual backflow.

SUMMARY AND CONCLUSIONS

Recent experiments on the mechanism of excitation of the sinual baro-receptors and on the sinual hemodynamics are discussed.

Normal stimulation of the Hering's nerve depends on the state of relaxation or contraction of the sinual wall. On the other hand, the muscle of this wall is controlled by a symphatetic-adrenergic mechanism. If the muscle is relaxed the sinual pressure determines reflexly the level of systemic pressure; if the muscle is contracted there are sinual baro-receptors continually stimulated and the systemic pressure is, up to a certain point not well defined, independent of the sinual pressure.

From the experiments on sinual hemodynamics it is concluded that following carotid occlusion the reduction of sinual pressure depends (in dogs) on two factors; drainage mainly through the external carotid artery and backflow mainly from the occipital artery. This backflow, in a large measure, seems not to affect the pressor reflex.

After common carotid occlusion, clamping of external carotids redu-

ces systemic hypertension, tachycardia, and reno —and spleno— contractions provoked by the pressor reflex.

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THE ACTION OF ALLOXAN IN THE TURTLE *PSEUDEMYS D'ORBIGNYI D* AND *B*

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THE ADMINISTRATION of alloxan to mammals causes marked destructive lesions of the beta cells of the islets of Langerhans of the pancreas, going from degranulation and vacuolization to necrosis. The blood sugar changes can be divided in three periods: an initial hyperglycemia, followed by a hypoglycemic phase, more or less marked according to the species, and a permanent diabetic stage if most of the beta cells are destroyed. In other words, alloxan diabetes is a pancreatic diabetes due to selective destruction of the beta cells.

In fish, hyperglycemia due to alloxan injection was observed in selacean (Saviano, 1947), and alloxan diabetes in the *Opsanus tau* (Lazarow and Berman, 1948). The islets of Langerhans showed selective necrosis with picnosis and shrinkage of the beta cells in the *Opsanus tau* (Lazarow and Berman, 1948); in the selacean there was marked development of the beta cells with an increase in the number of granules (Saviano, 1947). In some teleostei only changes in the shape of the beta cells were observed (Saviano, 1947), or nuclear picnosis, selective degranulation or cytolosis only in the beta cells (Grosso, 1950). In the toad *Bufo arenarum* Hensel, lesions of the islets were not observed (Houssay, Houssay and Sara, 1945; Biasotti and Porto, 1945). These animals showed the initial hyperglycemia and a large secondary hypoglycemic phase, lasting some days and without diabetic hyperglycemia (Houssay, Houssay and Sara, 1945). In snakes, hyperglycemia was observed, but without evident lesions of the beta cells (Saviano and de Franciscis, 1948). In

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the frog *Rana pipiens*, degranulation and vacuolization were observed, but no necrosis (Seiden, 1945).

Material and methods

Turtles *Pseudemis d'Orbignyi* of both sexes were used throughout the experiments, with a body weight between 800 and 1500 g, living in pools and eating fish. Before the injection of alloxan they were fasted for 48 hours, but since the date of their last feeding could not be precisely determined because they eat probably once every 3, 4 or more days, the period of fasting may be higher in some of the animals.

Subtotal pancreatectomy was perfomed with a technique similar to that for total pancreatectomy described by Foglia et al. (unpublished experiments).

Alloxan (Eastman Kodak), was injected into the jugular vein or the peritoneal cavity and in doses of 50, 150 or 200 mg per kg of body weight (the weight of the soft tissues in the turtles represents about one third of the total body weight). Blood was taken from the jugular vein and glucose measured with the Somogyi-Nelson method.

At the end of the experiment the animals were autopsied and the pancreas removed, fixed in Helly solution and stained with hematoxylin-eosin, Gomori's chromic hematoxylin and periodic acid leucofucsin (PAS), for glycogen investigation.

Results

The alloxan injected turtles lived, in general, in good conditions for weeks. One died after three days with a blood sugar of 336 mg per cent, and a few during the first five weeks. When they died, they were in poor condition with marked muscular hypotonia. Hypoglycemic convulsions were not observed. Autopsy revealed a red dark liver, the bladder full of urine and a general dilatation of the gut.

Glycemia. — The changes of the blood sugar were slow, as generally happens in poikilotherms. The initial hyperglycemia was observed, the hypoglycemic phase came afterwards lasting some days and, in two cases, diabetic hyperglycemia was seen after weeks.

The initial hyperglycemia was observed after 4 hours of the injection (2 animals), 9 hs (3), 1 day (2), 2 days (2), and 3 days (9); in other words, it was observed more frequently after 3 days and was not present in only one turtle. In some animals the hyperglycemia was very high (T_3:336 mg %, T_4:242 mg %, T_7:260 mg %, T_{13}:240 mg %).

Afterwards, glycemia begins to fall, since the second day in 4 cases, after the 4 th in 4 and after the 5 th day in 4 animals. Hypoglycemia was not observed in 4 turtles and was doubtful in one. The hypoglycemic phase lasted for a long time: 4 to 8 days in 2 cases, 16 to 19 days in 3, 26 days in 2 and 32 to 38 days in 5 animals. After 34 to 40 days of the injection of alloxan still 5 out 11 turtles had hypoglycemia.

Later on there was a rise of the blood sugar to almost normal values (2 cases), or to levels higher than normal (3 cases). In two of these last three turtles there was a late diabetes. In 5 out of 11 turtles there was no recovery from the hypoglycemic phase after 34 to 40 days.

PSEUDEMYS		Dose	GLYCEMIA (mg %)															
			Hours			Days												
Nº	Weight	mg/Kg	0	4	9	1	2	3	5	8	13	24	31	34	40	52	58	60
1 ♂	1.210	V·50	84	86	142		140		20	48	74	100		88 X		–	–	–
2 ♀	1.150	"	48	70	72		62		54	26	12	84		108		240	234	274 XY
3 ♂	1.300	P.150	56	72	86		258	336	-	-	-	–	–	–		–	–	–
4 ♂	850	"	94	104	86		242		134	124	136 X	–	–	–		–	–	–
5 ♂	1.060	"	108	166	124		182		156	84	140	240	122 XX	–		–	–	–
6 ♀	1.500	"	80	142	94		38		40	36	332	114		92		216	200	180 XX
7 ♂	1.540	P.200	72	240	254	240	248	260	170	142	114	58	44	74	56 XY			
8 ♂	1.250	"	150	146	168	150	142	174	194	214 X	–	–	–	–	–			
9 ♀	1.080	"	80	72	72	80	66	58	76	52	82	60	22	72	92 XX			
10 ♂	700	"	90	116	142	142	134	174	170	142	94	100	130 X	–	–			
11 ♂	950	"	72	96	96	100	88	118	92	40	58	58	22	48	68 XY			
12 ♂	1.350	"	138	120	124	146	110	108	66	50	66	60	34	16	58 XX			
13 ♂	1.180	"	142	150	130	206	192	240	124	72	42	122 X	-	–	–			
14 ♀	1.370	"	146	146	150	180	138	184	108	96	112	48	66	88	80 XX			
15 ♂	880	"	92	120	146	126	128	110	68	64	82	52	50	104	132 XX			
16 ♂	950	"	72	234	214	228	146	110	64	66	92	46 X	-	–	–			
17 ♂	1.000	"	116	120	116	142	88	148	72	66	68	48	52	62	58 XY			
18 ♂	1.100	"	112	96	96	130	142	160	104	68	60	54	44	84 X	–			
Average ± σ			98±28	128±49	128±48	156±48.8	141±63.5	168±75.1	101±51.2	82±48.6	98±78.7	83±50	59±37	76±26.4	197±13.1	228±16.9	217±24	227±66.4

CHART 1. — *Blood sugar values of alloxan injected turtles.*

X — *Spontaneous death.*

XX — *Dead by decapitation.*

Y — *Intravenous route.*

P — *Peritoneal route.*

In two animals there was a marked diabetic hyperglycemia with blood sugar values between 234 and 274 mg % and 180 to 216 mg %, 50 and 60 days after the injection of alloxan (chart 1).

The controls animals (Charts 2 and 3), normal turtles in which samples of blood sugar were taken at frequent intervals, during 6 days, showed that this procedure has no influence on the blood sugar levels.

Nº	GLYCEMIA (mg %)							
	Hours			Days				
	0	3	6	1	2	3	4	6
12	56±5.7	52±7.0	97±10.3	58±10.3	49±13.3	61±9.8	50±5.4	54±3.1

CHART 2. — *Blood sugar changes in normal turtles in which samples of blood were obtained at frequent intervals (average of 12 animals).*

Nº	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	T.M.
mg %	34	28	40	38	36	48	138	72	24	76	53.4

CHART 3. — *Blood sugar values of 10 normal turtles at autopsy.*

Histology of the pancreas. — Performed by Dr. A. F. Cardeza of the "Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina".

The pancreas of 9 turtles Pseudemys d'Orbignyi injected with alloxan were studied; in those from diabetic animals there were lesions of the islets of Langerhans of different intensity, and with Gomori's stain, only the beta cells were damaged. The lesions were more marked in the turtles 2 and 6, severe in 4 and 7 and less severe in 1 and 8. In these pancreas an increase of the size of the islets was observed as a result of a marked cellular tumefaction. At the same time there was a partial or total degranulation in the cytoplasm of the beta cells. In the cases of total degranulation, hydropic degeneration with positive reaction for glycogen (PAS), and nuclear margination were present. The reaction became negative after a previous digestion with ptialin (Fig. 1 and 2).

Alloxan in turtles with diabetes due to subtotal pancreatectomy. — In the *Bufo arenarum* Hensel the marked hypoglycemic effect of alloxan counteracts the hyperglycemic action of total pancreatectomy (Houssay, Houssay and Sara, 1945). In the turtle, in which alloxan produces an intense and quick hyperglycemia it does not counteract the rise of the blood sugar following partial pancreatectomy (Chart 4).

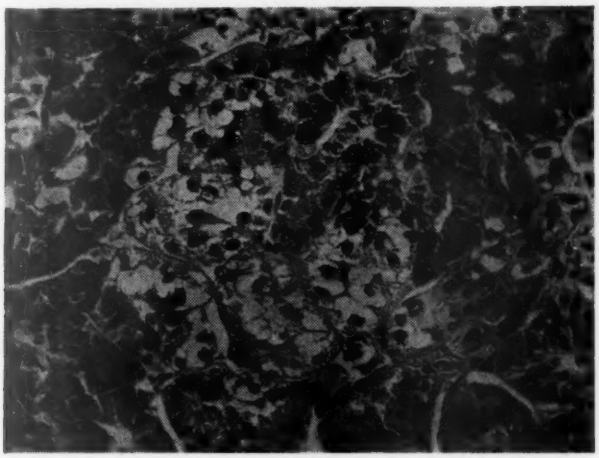

FIG 1. — *Islet of Langerhans of a turtle Pseudemys d'Orbignyi* (*T*$_r$*-17-VIII-54*). ***Tumefaction and vacuolization of the beta cells with nuclear picnosis. Hematoxylin-eosin. X 480.***

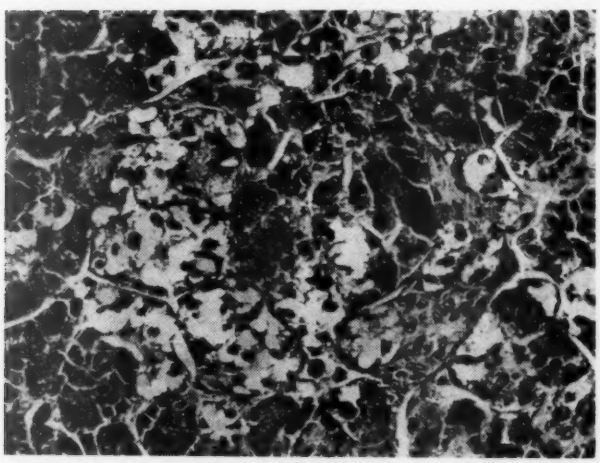

FIG 2. — *The same islet with Gomori's stain. Partial and total degranulation of the beta cells (alveolar aspect).*

In the diabetic animals an apparently reduced blood clotting time was observed, as has been previously reported (Lopes et. al., 1954).

Discussion

As in other poikilotherms, the blood sugar changes in turtles after

Nº	ALLOXAN		GLYCEMIA (mg %) - Hours			
	mg/kg	Via	0	24	48	72
1	200	Venous	72	400	×	
2	"	"	82	520	×	
3	"	"	46	420	400	×
4	"	"	100	470	×	
5	"	"	76	380	420	

Chart 4. — *Glycemia of partially depancreatized turtles injected with alloxan.*

the injection of alloxan, are slow. After the initial hyperglycemia, a secondary hypoglycemia is shown. We don't know if this is the result of an increase of insulin secretion or a higher output of glucose in peripheral tissues. Perhaps the action of alloxan in the liver plays a role, because in perfusion experiments, less output of glucose was observed in the toad *Bufo arenarum* Hensel (Houssay and Gerschman, 1947) and in the frog *Rana catesbiana* (Goldner and Hernandez-Jauregui, 1953).

After the secondary hypoglycemia the blood sugar rose back to normal or slightly above normal levels. Only in two animals, those with a longer survival time, a marked diabetic hyperglycemia was observed, after 50 to 60 days.

The lesions of the islets of Langerhans, seen in fish, batrachians and reptiles, are less marked than those observed in mammals. In our turtles no observations were made of the initial changes after alloxan administration, but lesions were observed in those animals sacrificed with hyperglycemia and specially in those who had a longer survival time and higher blood sugar levels.

The hydropic degeneration (glycogen infiltration), of the beta cells is similar to that observed in other diabetic animals. Perhaps in the turtles this is also an index of hypofunction of the beta cells, which is followed by a diabetic stage. But since the hyperglycemia produced in the turtle by the ingestion of glucose is also accompanied by hydropic vacuolization (unpublished experiments), these changes may be only a consequence of the hyperglycemia. In mammals, the beta cells infiltrated with glycogen show generally nuclear picnosis and shrinkage. This fact

and the coexistence of diabetes seems to indicate a suffering and hypofunction of the beta cells.

SUMMARY

Alloxan was injected in turtles Pseudemis d'Orbignyi and the following results were obtained: a) initial rise of the blood sugar of short duration; b) hypoglycemia lasting for days and c) recovery of the blood sugar to normal or slightly supernormal levels.

Two animals developed, after 52 and 60 days, a diabetic state, with blood sugar level between 180 and 274 mg %, when the mean values for normal turtles is 98 mg %.

The pancreas of the animals sacrificed with high values of glycemia showed a marked hydropic degeneration (glycogen infiltration) of the beta cells of the islets of Langerhans.

ACKNOWLEDGEMENTS

This work was supported by the "Conselho Nacional de Pesquisas do Brasil", and was carried out under the scientific direction of Dr. Ricardo R. Rodríguez. We wish to acknowledge the technical assistance of Dr. Marcelo Barros and Mr. Pedro P. Santos, the important contribution of Dr. Adolfo F. Cardeza, who performed the histological study the pancreas and the revision of the manuscript by Dr. Bernardo A. Houssay.

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ON THE MECHANISM OF ACTION OF BIS-(8'-QUINOLILOXY) 1.5-PENTANE DI-IODO ETHYLATE (3381 RP) ON THE NEURO-MUSCULAR JUNCTION *

F. HUIDOBRO

(Department of Pharmacology. Catholic University, Santiago de Chile)

THE BLOCKING action of bis-(8'-quinoliloxy) 1,5-pentane di-iodo-ethylate (3381 RP) on the neuro-muscular junction of the quadriceps muscle of cats under chloralose anesthesia was described in a previous publication (2). It was suggested that the paralysis induced by the drug might be due to polarization of the end-plate. This hypothesis was developed mainly because the action of the compound was counteracted by depolarizing agents (C-10) and also because its effect was different from that produced by *d*- tubocurarine; conversely, there was a post-tetanic decrement and the compound 3381 RP did not potentiate the block induced by *d*-tubocurarine.

Later, it was demonstrated (1,3) that the effects of the compounds causing neuro-muscular block may vary according to the synapsis being considered. Since a polarizing action was suggested to explain the intense neuro-muscular block, it was believed worthwhile to study the action of 3381 RP on other neuro-muscular preparations.

Experiments were perfomed in cats under the same anesthesia and using the same technique as described in an earlier publication (2) ; except that the tibialis anterior and soleus muscles were employed instead of the quadriceps.

It was demonstrated that when muscles were depressed by the action of 3381 RP, a tetanus produced an important "decurarization" (Fig. 1) a phenomenon that was completely different from the post-tetanic depression observed when the quadriceps was used (see fig. 1 of the earlier paper (2). A summation of the effects of the drug with those induced by *d*-tubocurarine was also observed.

Based on these facts, it is not proper to consider that compound 3381 RP induces neuro-muscular paralysis through a polarizing action, but rather that its mechanism of action is similar to that of *d*-tubocura-

* This work was carried out under support of the Gildemeister Foundation, Santiago de Chile.

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rine; namely by preventing the depolarizing action of acetylcholine. The difference between the action of the drug on the quadriceps and on the soleus and tibialis is another example of a drug having different effects, depending on the neuro-muscular synapsis employed.

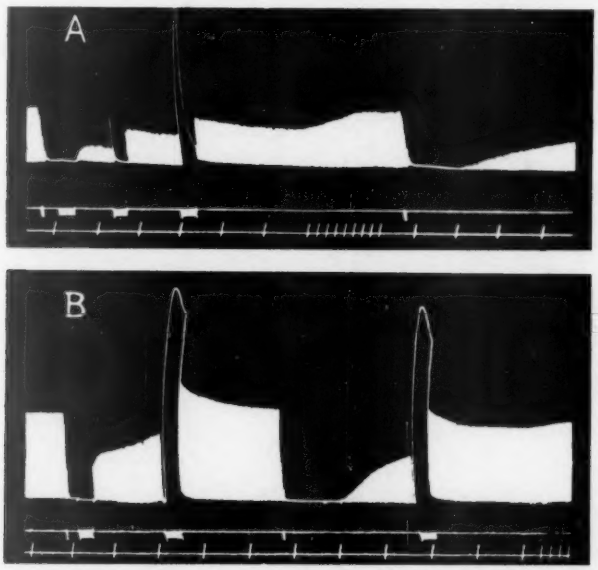

FIG. 1. — A: *Cat weighing 3.000 g. The soleus muscle was stimulated through the nerve, at a frequency of 15 stimuli per minute.*
Vertical line: 1 mg of 3381 RP intra-aortic.
Bar: stimulus of a frequency of/15 minute was replaced by another of 64/second.
B: *Cat weighing 4.400 g. The tibialis muscle was stimulated through the nerve, at a frequency of 15 stimuli per minute.*
Vertical line: 1 mg of 3381 RP intra-aortic.
Bar: stimulus of a frequency of/15 minute was replaced by another of 64/second.
Time: in A and B, minutes. In both cases, the different distances of time are due to changes of speed in the kymograph.

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PROCEEDINGS OF THE "SOCIEDAD ARGENTINA DE BIOLOGIA"

Buenos Aires, April 21st, 1955

ARTERIAL HYPERTENSION IN HEMIDECORTICATE RATS

M. R. COVIAN AND H. E. J. HOUSSAY

Removal of one cerebral hemisphere in the rat produces arterial hypertension, after a latence of 2 to 20 days (77 % of cases). The hypertension lasts 30-60 days in 90 % of the rats and 61-80 days in 10 %. The hypertension was observed in 92 % of the 72 operated rats. The blood pressure rises from 112 mm Hg to 145 mm Hg (average, range 130-190 mm Hg).

The blood pressure fell more in decorticate rats than in normal ones by action of dibenamine (10 mg/100 g, per os), F 933 (0.5 mg/100 g, subcutaneous injection), tetraethylammonium bromide (0.5 mg/100 g, injection) Hydergine (0.3 mg/100 g, injection). After adrenalectomy the blood pressure of hypertensive rats returns permanently to normal level.

Apparently, the hypertension is related to an increase of sympathetic tone. It can be due to traumatic stimulation or to release of an inhibitory cortical influence on hypothalamus and vasomotor centers. The last explanation is the more probable because the animals have also piloerection, motor hyperactivity, hyperexcitability and hyperphagia.

(Instituto de Biología y Medicina Experimental. Costa Rica 4185, Buenos Aires)

This paper will appear "in extenso" in "Circulation Research".

HYPOPHYSIS AND HAIR GROWTH IN THE WHITE RAT

A. B. HOUSSAY AND J. C. PENHOS

Growth of hair in adult rats is cyclic, every hair follicle has alternate periods of growth and rest. Hair growth is not uniform, but there are centers of growth from which it spreads in waves all over the body.

Removal of the pituitary greatly accelerates hair growth, in rats, immediately after the operation. All the hair follicles are intensely stimulated simultaneously and the whole denuded area is rapidly covered by a normal coat of hair growing all at the same time.

This growth of hair follows the same pattern and takes exactly the same time in covering the denuded area in hypophysectomized rats, adrenalectomized rats and hypophysectomized-adrenalectomized rats.

Gonadectomy does not change the rate of hair growth in hypophysectomized rats or adrenalectomized rats.

When the hypophysectomy or the adrenalectomy is not complete the characteristic pattern of accelerated hair growth is not present.

The growth of hair in hypophysectomized rats is inhibited by the adrenocorticotrophic hormone of the pituitary or by chorionic gonadotrophins but not by the growth hormone of the pituitary.

Hair cycle in rats and mice is controlled by endocrine factors, the pituitary being the principal one.

(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires)

This paper will appear "in extenso" in the "Revista de la Sociedad Argentina de Biología".

DIABETOGENIC ACTION OF PROLACTIN

B. A. HOUSSAY, E. ANDERSON, R. W. BATES AND CH. H. LI

A preparation of Prolactin (25 units per mg) prepared by Squibb, (nº 71713) has produced diabetes in 7/11 dogs with reduced pancreatic mass (extirpation of 76 to 84 %). The active daily dosis (1 to 5.7 mg per kg per day) was injected 4-5 days. The diabetes was obtained in dogs without thyroid or without hypophysis.

In 6/8 cats with reduction of pancreatic mass (extirpation of 61

to 87 %) the same preparation produced diabetes (5.9 to 10.8 mg per kg per day of the preparation 71713).

A purest prolactin prepared by Li and Cole has produced diabetes in 3 dogs with 1 to 5 mg per kg per day. Another sample has not produced diabetes in 4 dogs with 5 mg per kg per day, in 4 days.

The prolactin was more active than a pure corticotrophin in 4 dogs. Prolactin was in all animals definitely less active than the growth hormone.

The next step will be to try to elucidate if the action is due to prolactin alone or entirely to a contaminant or to an association of both substances.

(Instituto de Biología y Medicina Experimental. Costa Rica 4185, Buenos Aires)

This paper will appear "in extenso" in the "Revista de la Sociedad Argentina de Biología".

INHIBITION OF ABSORPTION OF GONADOTROPHINS BY MICROCRYSTALS OR CALCIUM IN THE TOAD

J. C. PENHOS

Injection into the dorsal lymph sac of 0.75-0.5 mg of *pars distalis* of hypophysis of *Bufo arenarum* Hensel produces ovulation in the female toad. This action was inhibited when microcrystals of Progesterone or estradiol benzoate or Cholesterol (1 mg of each) were injected the same as the gonadotrophin into the dorsal lymph sac. The inhibition is probably due to a reduced absorption of the gonadotrophin from the dorsal sac into the blood via the lymph hearts, because ovulation was not inhibited when the microcrystals and the *pars distalis* were injected separately (one subcutaneously and the other in the peritoneum).

The same type of inhibition of absorption was demonstrated in male toads when $CaCl_2$ (150 mg) was injected under the skin together with 0.05-0.04 mg of *pars distalis* of hypophysis of *Bufo arenarum* Hensel. Spermiation occurred when the $CaCl_2$ and the gonadotrophins were injected in different territories (one subcutaneously and the other in the peritoneal cavity).

(Instituto de Biología y Medicina Experimental. Costa Rica 4185, Buenos Aires)

This paper "in extenso" will appear in the "Revista de la Sociedad Argentina de Biología".

NORMAS PARA LA PRESENTACION DE TRABAJOS

Los trabajos deben ser enviados al Jefe de Redacción del país de origen. Si no existiera Comité Editorial, deberán ser enviados a la Secretaría de Redacción de Acta Physiologica Latinoamericana, Avda. R. Sáenz Peña 555, Buenos Aires, Argentina. La revista no se responsabiliza por los daños sufridos por el manuscrito o por su pérdida. Se recomienda a los autores conservar una copia completa de los trabajos que envíen por correo.

Se aceptan artículos originales inéditos o que hayan sido publicados previamente en forma parcial o completa en alguna revista local.

Los trabajos deberán ser escritos a máquina en papel tamaño carta, no transparente, a doble espacio y con amplio margen. Las ilustraciones deberán estar numeradas (fig. 1, fig. 2, etc.), y llevar al pie una leyenda clara y concisa. Las fotografías hechas en papel brillante, nítidas. Los gráficos y diagramas, dibujados con tinta china sobre fondo blanco, listos para reproducir.

Se publicarán trabajos escritos en castellano, portugués, francés o inglés. Los que estén escritos en castellano o portugués deberán contener al final un resumen en inglés.

Las citas bibliográficas se harán en el texto mediante números [por ej.: algunos autores (2, 15) y en especial Jones (5)] o autores y año [por ej.: (Breslauer, 1919)]. Al final del trabajo la bibliografía se ordenará alfabéticamente y con numeración progresiva, en el primer supuesto, y alfabéticamente en el segundo. Para las abreviaturas de las revistas, etc., se seguirán las recomendaciones del World List of Scientific Periodicals. La disposición de tales citas debe ajustarse a los ejemplos siguientes:

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(2) Goldberger, E.: *Unipolar lead electrocardiography*, Philadelpia, Lea and Febiger, 1947.

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Se exponen a continuación algunas abreviaturas comunes:

metro	m	litro	l	microgramo	µg
centímetro	cm	centímetro cúbico	cm^3	gama	γ
milímetro	mm	mililitro	ml	por ciento	%
micrón	µ	kilogramo	kg	hora	h
milimicrón	mµ	gramo	g	minuto	m
Ansgtröm	Å	miligramo	mg	segundo	s
				milisegundo	ms

Para evitar la confusión derivada de la notación decimal diferente según los países, se adopta el punto decimal y se suprime toda notación entre millares sustituyéndose por un espacio: 10 000 (no 10.000 ni 10,000) —0.90 (no 0,90).

SUMARIO

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